

USER GUIDE

**ion torrent**  
*life* technologies

# Ion PGM<sup>™</sup> Hi-Q<sup>™</sup> Sequencing Kit

for use with:

Ion PGM<sup>™</sup> System

Ion 318<sup>™</sup> Chip v2

Ion 316<sup>™</sup> Chip v2

Ion 314<sup>™</sup> Chip v2

**Catalog Number** A25592

**Publication Number** MAN0009816

**Revision** B.0

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# About this guide

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**IMPORTANT!** Before using this kit with the Ion PGM™ System, read and understand the information in Appendix G, “Safety”.

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## Revision history

Revision	Date	Description of change
B.0	4 September 2014	Full release version
A.0	6 December 2013	Technology access version

## Purpose of the guide

The *Ion PGM™ Hi-Q™ Sequencing Kit User Guide* (Pub. no. MAN0009816) provides protocols and reference information for using the Ion PGM™ Hi-Q™ Sequencing Kit with Ion 314™, Ion 316™, and Ion 318™ Chips on the Ion PGM™ System.



# Product information

## Product description

The Ion PGM™ Hi-Q™ Sequencing Kit (Cat. no. A25592) includes reagents and materials for sequencing up to ~400-bp inserts using the following chips on the Ion PGM™ System:

- Ion 314™ Chip v2
- Ion 316™ Chip v2
- Ion 318™ Chip v2

The kit includes components for cleaning and initializing the instrument. The number of sequencing runs per kit depends on the length of the base reads, as shown in the table below (see Appendix D, “Sequencing run times” for more information).

Sequencing type	Number of flows	Number of runs per kit	Maximum number of runs per initialization <sup>[1]</sup>
400-base read	850	4	1
200-base read	500	8	2
≤100-base read	260	12	3

<sup>[1]</sup> For best results, begin the run within 1 hour after initialization.

## Intended use

The Ion PGM™ Sequencer performs real-time measurements of hydrogen ions produced during DNA replication.

## Library kit compatibility


This sequencing kit can be used with all library types prepared using any Ion library kit.

## Template kit compatibility

We recommend the following template preparation kits for optimal performance:

- Ion PGM™ Template OT2 400 Kit (Cat. no. 4479878)
- Ion PGM™ Template OT2 200 Kit (Cat. no. 4480974)

## Software compatibility

The instruments and kits described in this guide are designed for use with Version 4.2 or later of the Torrent Suite™ software and Ion PGM™ System software. To view the current software version, log in to the Ion PGM™ Torrent Browser, click on the  "Gear" tab, then select **About**.

To update the software, see “Update the Ion PGM™ Sequencer software” on page 78.

Version-specific information about the software is provided in the software Release Notes.

## Ion PGM™ Chip compatibility

This sequencing kit is compatible with the Ion 314™ Chip v2 (Cat. no. 4482261), Ion 316™ Chip v2 (Cat. nos. 4483188 and 4483324), and Ion 318™ Chip v2 (Cat. nos. 4484354 and 4484355).

## Ion PGM™ Hi-Q™ Sequencing Kit contents and storage

The Ion PGM™ Hi-Q™ Sequencing Kit (Cat. no. A25592) includes the following components:

Ion PGM™ Sequencing Supplies (Part no. A25587)				
Component	Color	Quantity	Storage	
Wash 1 Bottle w/ label (250 mL)	Green	1 bottle	15°C to 30°C	
Wash 3 Bottle w/ label (250 mL)	Green	1 bottle		
Ion PGM™ Reagent Bottle Sipper Tubes	Blue	16 tubes		
Ion PGM™ Wash Bottle Sipper Tubes	Gray	8 tubes for 250-mL bottles 4 tubes for 2-L bottles		
Reagent Bottles w/ labels (50 mL)	—	25 bottles		
Ion PGM™ Hi-Q™ Sequencing Reagents (Part no. A25588)				
Component	Cap Color	Quantity	Volume	Storage
Ion PGM™ Hi-Q™ Sequencing Polymerase	Yellow	1 tube	36 µL	-30°C to -10°C
Sequencing Primer	White	1 tube	144 µL	
Control Ion Sphere™ Particles	Clear	1 tube	60 µL	

Ion PGM™ Hi-Q™ Sequencing Solutions (Part no. A25589)				
Component	Label	Quantity	Volume	Storage
Ion PGM™ Hi-Q™ Sequencing W2 Solution	Black	4 bottles	126.25 mL	2°C to 8°C (store W2 Solution protected from light)
Ion PGM™ Cleaning Tablet	—	4 tablets	—	
Annealing Buffer	—	1 bottle	12 mL	
Ion PGM™ Sequencing W3 Solution	—	2 bottles	100 mL each	

Ion PGM™ Hi-Q™ Sequencing dNTPs (Part no. A25590)				
Component	Cap Color	Quantity	Volume	Storage
Ion PGM™ Hi-Q™ Sequencing dGTP	Black	1 tube	80 µL	-30°C to -10°C
Ion PGM™ Hi-Q™ Sequencing dCTP	Blue	1 tube	80 µL	
Ion PGM™ Hi-Q™ Sequencing dATP	Green	1 tube	80 µL	
Ion PGM™ Hi-Q™ Sequencing dTTP	Red	1 tube	80 µL	

## Ion PGM™ Wash 2 Bottle Kit

The Ion PGM™ Wash 2 Bottle Kit (Cat. no. A25591) is sold separately, and includes the following components:

Component	Quantity	Volume	Storage
Wash 2 Bottle w/ label (2 L) <b>Note:</b> Must be conditioned at least 8 hours before use as described in "Condition the Wash 2 Bottle for first use" on page 22	1 bottle	—	15°C to 30°C
Wash 2 Bottle Conditioning Solution	1 bottle	125 mL	

## Ion Chip Kits

The following Ion Chip Kits are compatible with this sequencing kit, and are sold separately:

Component	Quantity	Catalog no.	Storage	
Ion 318™ Chip Kit v2	4 pack	4484354	15°C to 30°C	
	8 pack	4484355		
Ion 316™ Chip Kit v2	4 pack	4483188		
	8 pack	4483324		
Ion 314™ Chip Kit v2	8 pack	4482261		

## Instruments and server

This sequencing kit is designed for use with the following instruments and server, ordered separately.

Components	Catalog no.
Ion PGM™ System and accessories	4462921
Ion PGM™ Torrent Server	4462918
Ion PGM™ Chip Minifuge:	
120 VAC	4479672
230 VAC	4479673

## Required materials and equipment

This kit uses common molecular biology equipment, supplies, and reagents. MLS: Fisher Scientific ([www.fisherscientific.com](http://www.fisherscientific.com)) or other major laboratory supplier. Life Technologies website: [www.lifetechnologies.com](http://www.lifetechnologies.com).

**Note:** The procedures in this guide have been validated using these specific materials. Substitution may adversely affect system performance.

Description	Supplier	Catalog number	Quantity
Tank of compressed nitrogen (grade 4.5, 99.995% or better)	MLS	Varies	1
Multistage (dual-stage) gas regulator (0-50 PSI, 2-3 Bar output)	VWR International	55850-422	1

Description	Supplier	Catalog number	Quantity
<b><i>Choose from one of the following systems:</i></b> ELGA® PURELAB® Flex 3 Water Purification System Equivalent 18-MΩ water purification system	Life Technologies MLS	4474524 Varies	1
Microcentrifuge (capable of >15,500 × g, fits 1.5-mL and 0.2-mL microcentrifuge tubes)	MLS	Varies	1
0.22-μm or 0.45-μm vacuum filtration system and filters (nylon or PVDF filters, 1-L volume)	MLS	Varies	1
Rainin® Pipet-Lite® XLS with RFID LTS 10 to 100 μL <sup>[1]</sup> (Alternatives from Gilson and Eppendorf may be used)	Rainin Gilson Eppendorf	SR-L200F F167203 022493030	1
Rainin® Pipet-Lite® XLS with RFID LTS 2 to 20 μL <sup>[1]</sup> (Alternatives from Gilson and Eppendorf may be used)	Rainin Gilson Eppendorf	L-20XLS F21023 022470159	1
Rainin® pipette tips (Alternatives from Gilson and Eppendorf may be used)	Rainin Gilson Eppendorf	SR-L200F F167203 022493030	1 case
Rainin® barrier pipette tips <sup>[1]</sup> (Alternatives from Gilson and Eppendorf may be used)	Rainin Gilson Eppendorf	SR-L10F F171303 022493028	1 case
PCR tubes, Flat Cap, 0.2-mL (do not use polystyrene tubes)	VWR	10011-780	1 box
Vortexer with a rubber platform	MLS	Varies	1
Thermal cycler with a heated lid	MLS	Varies	1
Graduated cylinders (1 L or 2 L volume)	MLS	Varies	1
Glass bottle (1 L)	MLS	Varies	1
15-mL conical tubes	MLS	Varies	Varies
NaOH (10 M), molecular biology grade	MLS	Varies	Varies
Pipette set and filtered tips, P2, P20, P200, and P1000 μL	MLS	Varies	1 set
Microcentrifuge tubes, 1.5-mL or 1.7-mL	MLS	Varies	Varies

Description	Supplier	Catalog number	Quantity
Syringe, 10 CC, Female Luer Lock (used for clearing lines)	Provided with the Ion PGM™ Sequencer, or MLS		1
Ion PGM™ 2.5 L Waste Bottle <sup>[2]</sup>	Life Technologies	4482565	1

Optional materials			
Ion PGM™ Controls Kit v2 <sup>[3]</sup>	Life Technologies	4482010	1
Ion PGM™ Sequencing Sippers Kit <sup>[4]</sup>	Life Technologies	4478682	1
Uninterruptable Power Supply (UPS) <sup>[5]</sup>	MLS	Varies	1

Materials needed if manual adjustment of W2 Solution pH is required (see Appendix C, "Manually Adjust W2 pH")			
Orion® 3-Star Plus pH Benchtop Meter Kit with electrode, electrode stand, and calibration buffers (or equivalent)	Thermo Scientific	1112003	1
Magnetic stirrer (must hold 2-L bottle)	MLS	Varies	1
Magnetic stir bar (4 cm)	MLS	N/A	1
1 N HCl	MLS	N/A	Varies

<sup>[1]</sup> Ensure tips from any vendors are low binding tips. Required for loading samples onto the Ion PGM™ Chips.

<sup>[2]</sup> Required one-time purchase for older ("Blue") versions of the Ion PGM™ System.

<sup>[3]</sup> Not commonly needed, but available for troubleshooting.

<sup>[4]</sup> Contains additional sipper tubes; not commonly needed.

<sup>[5]</sup> We recommend using an uninterruptable power supply (UPS) for laboratories that experience frequent power outages or line voltage fluctuations. The UPS must be compatible with 1500 W output or higher. The 1500 VA unit from APC provides ~11 minutes of backup power for an Ion PGM™ System.

## Ion PGM™ System with Reagent and Wash Bottles attached



Label	Component
A	Touchscreen
B	Chip clamp
C	Grounding plate
D	Power button
E	Reagent bottles
F	Wash 1 Bottle (W1 position)
G	Wash 2 Bottle (W2 position)
H	Wash 3 Bottle (W3 position)
I	Waste Bottle



# Before you begin

## Precautions before using the Ion PGM™ System

For additional safety information, see Appendix G, “Safety”.

### Update the software

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**IMPORTANT!** Before proceeding, make sure that you have updated the Torrent Suite™ and Ion PGM™ System software to Version 4.2 or later. See “Update the Ion PGM™ Sequencer software” on page 78.

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### Instrument installation by trained personnel only

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**IMPORTANT!** The Ion PGM™ System is installed by trained service personnel and must not be relocated without assistance from trained service personnel. See “Customer and technical support” on page 86.

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### Nucleic acid contamination

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**IMPORTANT!** A primary source of contamination is DNA fragments from previously processed samples. Do not introduce amplified DNA into the library preparation laboratory or work area.

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**IMPORTANT!** Possible contamination can occur during the transfer of dNTPs into Reagent Tubes. Be careful to avoid cross contamination of dNTP stocks. Barrier tips are required for all pipetting steps. Change gloves after handling concentrated dNTP stocks.

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### CO<sub>2</sub> contamination

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**IMPORTANT!** Dry ice (solid CO<sub>2</sub>) must be kept away from areas where buffers, wash solutions, or sources of molecular biology grade water for the Ion PGM™ System are used. High air concentrations of subliming CO<sub>2</sub> may change the pH of such buffers during or after their preparation. The stability of the pH of these buffers is a critical factor in the performance of the Ion PGM™ System.

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**Instrument vibration and clearances**


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**IMPORTANT!** Significant vibration during sequencing may add noise and reduce the quality of the measurements. The Ion PGM™ System must be installed on a bench that is free from vibrations or in contact with equipment that can cause vibrations to the bench (freezers, pumps, and other similar equipment)

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**IMPORTANT!** Position the Ion PGM™ System so that the front bezel is a minimum of 12 in. (30.5 cm) and the Reagent Tubes containing dNTPs are a minimum of 8 in. (20.3 cm) from the front of the laboratory bench. Place the instrument at least 40 in. (1 meter) away from major sources of electronic noise such as refrigerators or microwaves.

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**Static electricity**



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**IMPORTANT!** To avoid possible damage to the chip from static electricity, you must ground yourself on the grounding plate (located next to the chip clamp) prior to handling chips, and do not place chips on non-grounded surfaces such as a bench. Always use the grounding plate to hold chips that are not in the package or inserted in the chip clamp or the Ion PGM™ Chip Minifuge bucket.

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**Ventilation requirements**


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 **WARNING!** Instrumentation must be installed and operated in a well-ventilated environment as defined as having a minimum airflow of 6–10 air changes per hour. Assess the need for ventilation or atmospheric monitoring to avoid asphyxiation accidents from inert gases and/or oxygen depletion, and take measures to clearly identify potentially hazardous areas through training or signage. Please contact your Environmental Health and Safety Coordinator to confirm that the instruments will be installed and operated in an environment with sufficient ventilation.

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**Procedural guidelines**

- Use good laboratory practices to minimize cross-contamination of products. When designing the laboratory layout, consider the need for space separation of pre- and post-amplification activities. Dedicate laboratory supplies and/or equipment to the appropriate space to significantly reduce the potential for contamination.
- Pipet viscous solutions slowly and ensure complete mixing.
- Ensure all reagents are completely thawed at room temperature, i.e., no ice crystals are visible.
- Vortex all reagents, *except* for enzymes, for 5 seconds (enzymes should be mixed by flicking the tube with your finger four times). Pulse spin in a microfuge for 3–5 seconds before use.
- **Ion OneTouch™ Instrument only:** Always change gloves after handling waste oil, used amplification plates, and cleaning adapters.
- **Ion PGM™ Sequencer only:** When performing two sequencing runs from the same initialization, the second run must be started within 27 hours after initialization.

## Chip handling and use guidelines

- Always remove gloves before transferring chips on or off the instrument. Hold chips by their edges when handling.
- To avoid damage due to electrostatic discharge (ESD), **do not place chips directly on the bench or any other surface**. Always place chips either on the grounding plate on the Ion PGM™ Sequencer or in the Ion PGM™ Chip Minifuge bucket.
- Used chips cannot be reused for sequencing. Used chips may be marked for cleaning and initialization.

## Gas cylinders

You must supply the required nitrogen gas cylinder and accessories for the installation. This instrument requires a pressurized house line, or one size 1-A nitrogen gas cylinder that holds approximately 7.2 m<sup>3</sup> (257 ft<sup>3</sup>) of gas when full. Use only prepurified nitrogen of 99.995% (grade 4.5) or greater purity.



**CAUTION!** Damage to the instrument and its products can result from using impure gas, gases other than nitrogen, or an inadequate amount of gas.



**WARNING! EXPLOSION HAZARD.** Pressurized gas cylinders are potentially explosive. Always cap the gas cylinder when it is not in use, and attach it firmly to the wall or gas cylinder cart with approved brackets or chains.



**WARNING!** Gas cylinders are heavy and may topple over, potentially causing personal injury and tank damage. Cylinders should be firmly secured to a wall or work surface. Please contact your environmental health and safety coordinator for guidance on the proper installation of a gas cylinder.

### Perform a leak test

To perform a leak test on the gas cylinder:

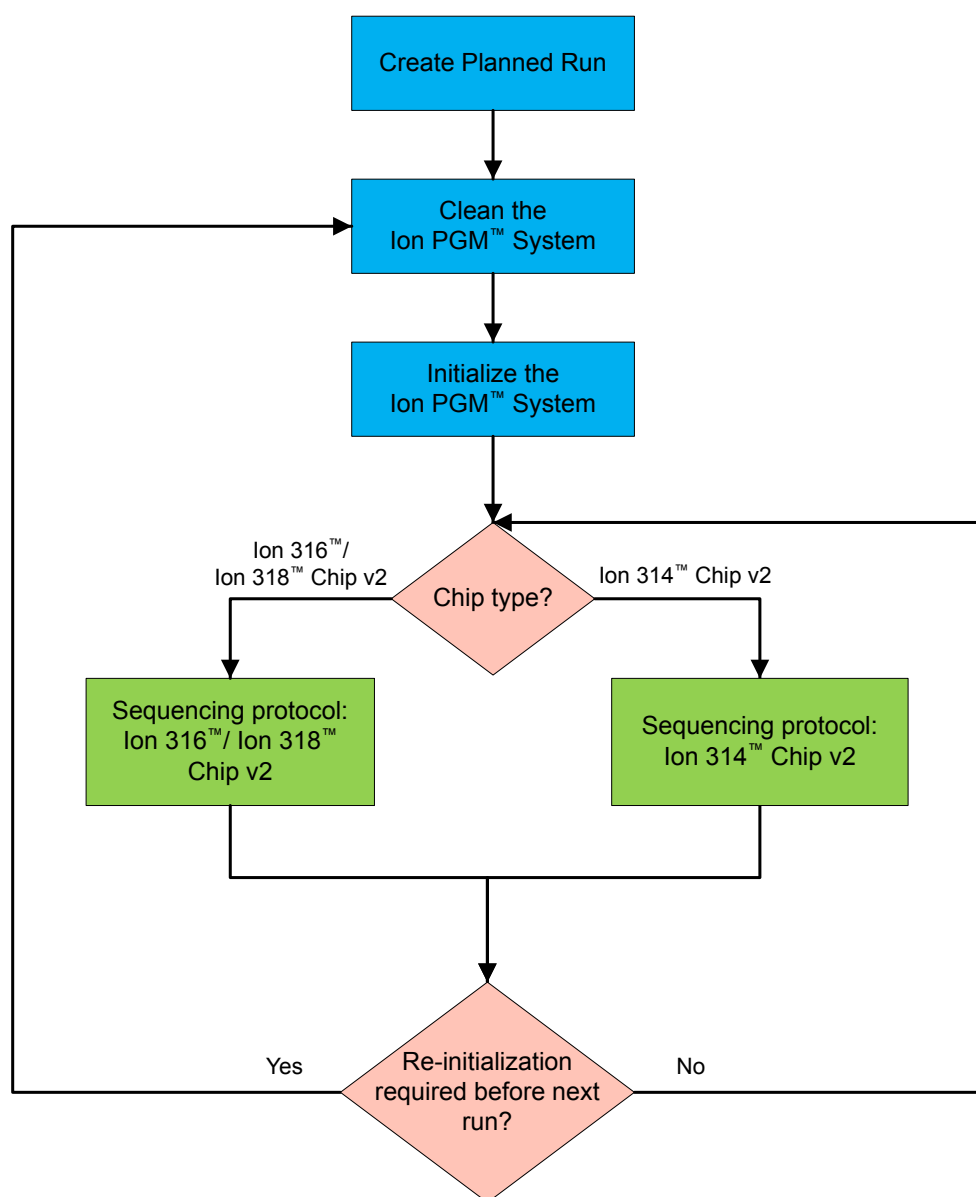
1. Open the main tank shutoff valve. The gas tank regulator's high-pressure gauge should read approximately 2000–2500 psi for a full tank.
2. Adjust the pressure to the instrument by slowly turning the pressure adjustment valve clockwise until the low-pressure gauge reads 30 psi.
3. Close the needle valve, then close the main tank valve.
4. Monitor the high-pressure gas tank regulator gauge for 5 minutes. There should be no noticeable drop in pressure.

If the pressure...	Then...
Drops in 5 minutes	There may be a leak at either the needle valve or the gas tank regulator itself. Check the fittings and resolve any problems, then continue with the following steps.
Does not drop in 5 minutes	The instrument passes the leak test. Re-open the main tank valve and skip the following steps.

5. Open the main tank valve and the needle valve for at least 15 seconds to pressurize the instrument.
6. Close the main tank valve.
7. Monitor the high-pressure gas tank regulator gauge. There should be no more than a 100-psi drop in pressure after 5 minutes. Locate and resolve any leaks. Turn the main tank valve back on.

## Protocol workflow

The Ion PGM™ System uses the following workflow when performing sequencing runs:



# 3

## Create a Planned Run (required)

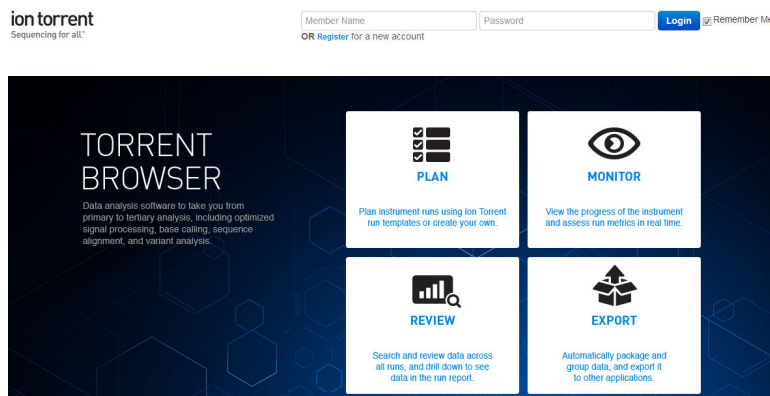
**IMPORTANT!** Before proceeding, make sure that you have updated the Torrent Suite™ and Ion PGM™ System software to Version 4.2 or later.

Planned Runs contain all the settings used in a sequencing run, including application type (gDNA, RNA, Ion AmpliSeq™, etc.), kits used, number of flows, barcodes (if any), and reference file.

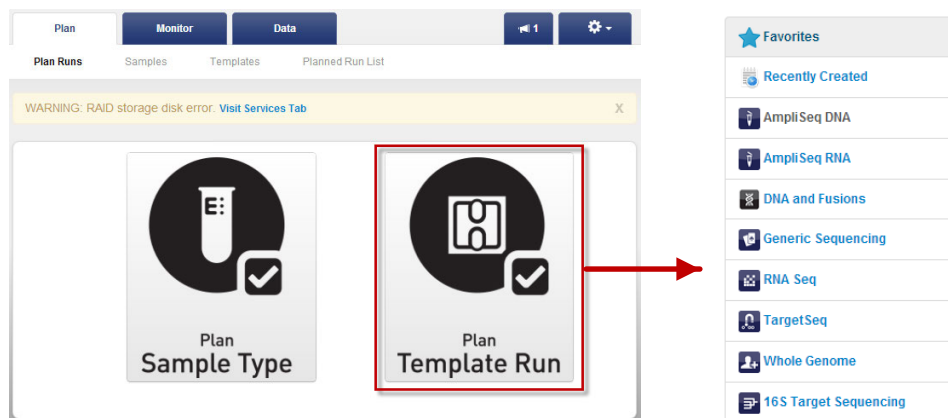
You create and save Planned Runs in the Ion PGM™ Torrent Browser, and then select the plan in the Ion PGM™ Sequencer touchscreen as part of the Run workflow, as described in the sequencing protocol for your chip type later in this guide.

For additional information, see the *Torrent Browser User Interface Guide*, available on the Ion Community at <http://ioncommunity.lifetechnologies.com>.

1. To create a Planned Run, log into the Torrent Browser for the Torrent Server connected to your Ion PGM™ System.



2. Select the **Plan** tab, select **Plan Template Run**, and locate the type of experiment you want to perform (e.g., Ion AmpliSeq™ DNA, DNA and Fusions, Generic Sequencing, etc.).



3. To plan a new run for that experiment type:
  - Click **Plan New Run** to create a new run without an existing template, or
  - Select an existing template from the list, click on the **Gear button** (⚙️) for that template, and select **Plan Run** to create a run from that template



- In the wizard, review each screen and make your selections. On the **Kits** screen, select the appropriate library, template, and sequencing kits, as well as chip and other information:

**Select the sequencing kits and then hit next.**

Sample Preparation Kit (optional):

Library Kit Type: [Details +](#)

Template Kit ☒ OneTouch ☐ IonChef:

Sequencing Kit:

Control Sequence (optional):

Chip Type (required):

Barcode Set (optional):

☒ Mark as Duplicates Reads ☐ :

☒ Enable Base Recalibration ☒ :

☒ Enable Realignment ☐ :



Flows :

**Note:** For a complete description of each field, see the *Torrent Browser User Interface Guide*.

- We recommend using the default 850 flows for 400-base-read sequencing with the Ion PGM™ Hi-Q™ Sequencing Kit. Otherwise, see Appendix D, “Sequencing run times” for a table listing the number of flows for different read lengths.
- When you have completed your selections, click on **Plan Run** at the end of the workflow. The plan will appear listed on the **Planned Runs** page under the name you specified.

**Planned Runs**

All | [by Template](#) | [by Sample](#)

<input type="checkbox"/> Select	Run Code	Run Plan Name ▲	Barcodes	Application	Project	Sample	Last Modified ▼	Status
<input type="checkbox"/>	0R009	test1				aaa	2013/10/30 10:54 AM	planned 

- You can then select the plan when you are setting up the run on the Ion PGM™ Sequencer (see “Select the Planned Run and perform the run” on page 41).



# Clean and initialize

## Condition the Wash 2 Bottle for first use

New Wash 2 Bottles must be conditioned with Wash 2 Bottle Conditioning Solution for at least 8 hours before first use. The Wash 2 Bottle Kit (cat. no. A25591) includes both a Wash 2 Bottle and Conditioning Solution.

**Note:** If necessary, you can reuse an existing Wash 2 Bottle while you condition a new bottle. Bottles can be used for sequencing up to 40 times before they must be replaced.

To condition the Wash 2 Bottle:

1. Fill the bottle to the mold line with 18 M $\Omega$  water, add the entire container of Wash 2 Bottle Conditioning Solution, then cap the bottle and invert it five times to mix.
2. Allow the bottle to sit at room temperature for at least 8 hours and preferably overnight, then dispose of the contents. The bottle is now ready for use.

## Cleaning

### Materials required

- 18 M $\Omega$  water (e.g., the ELGA<sup>®</sup> PURELAB<sup>®</sup> Flex Water Purification System)
- Cleaning bottles and collection trays (provided with the Ion PGM<sup>™</sup> System)
- Old chip that has been used for sequencing, marked for cleaning
- Used sipper tubes (from the previous run)
- Squirter bottle
- **Chlorite cleaning:** Ion PGM<sup>™</sup> Cleaning Tablet (provided in the kit)
- **Chlorite cleaning:** 1 M NaOH, diluted fresh each week from 10 M NaOH
- **Chlorite cleaning:** Glass bottle (1 L)
- **Chlorite cleaning:** 0.22- $\mu$ m or 0.45- $\mu$ m vacuum filtration system and filters

## Cleaning schedule

The Ion PGM™ Sequencer requires cleaning with either 18 MΩ water or a chlorite solution every time the instrument is initialized.

Clean with...	Schedule
18 MΩ water	<ul style="list-style-type: none"> <li>Daily, when instrument is in use (e.g., not necessary on weekends)</li> <li>After ≤1100 flows</li> <li>If more than 27 hours but less than 48 hours have elapsed between the last cleaning/initialization and the start of a run</li> <li>If you cleaned with chlorite a week ago and have not used the instrument since then</li> </ul>
Chlorite solution	<ul style="list-style-type: none"> <li>Once a week, unless the instrument has not been used since the last chlorite cleaning (in which case, clean with 18 MΩ water before using)</li> <li>If the instrument has been left with reagents for more than 48 hours (for example, over the weekend)</li> </ul>

## Cleaning setup

**IMPORTANT!** For all the following steps, use 18 MΩ water directly from the purification system. Do not use water that has been collected or stored in any other containers.

- Remove any wash and reagent bottles that are attached to the Ion PGM™ System before cleaning.
- Do not remove old sippers before cleaning. The sippers are used as part of the cleaning procedure.
- Old chips that have been used for sequencing can be marked and used in the cleaning procedure.
- Wash bottles (250 mL and 2 L) provided as part of instrument installation can be marked and used for cleaning. After you have used the wash bottles provided with the sequencing kit for the specified number of runs, you can use them as extra cleaning bottles. Mark them for cleaning use only.

## 18 MΩ water cleaning

- Empty any remaining solution from each cleaning bottle (two 250-mL bottles and one 2-L bottle) and rinse each bottle twice with ~100 mL of 18 MΩ water.
- Press **Clean** on the touchscreen, and select the **18-MOhm water cleaning** checkbox. Press **Next**.
- Using ungloved hands, secure a used chip designated for cleaning in the chip clamp.

**IMPORTANT!** Always make sure that both red rubber gasket port fittings are securely in place when securing chips with the chip clamp. Failure to do so can result in a spill hazard and instrument damage.

- Remove all wash and reagent bottles attached to the instrument. Keep the sippers in place at all positions. Press **Next**.
- Add 250 mL of 18 MΩ water to an empty 250-mL cleaning bottle.

6. Rinse the outside of the sipper tube in the W1 position on the instrument with a squirt bottle containing 18 MΩ water.
7. Attach the 250-mL bottle containing 18 MΩ water to the W1 position, ensuring that the W1 cap is screwed on tightly. Press **Next**.
8. Place the empty 2-L cleaning bottle in the W2 position and the empty 250-mL bottle in the W3 position, and insert the sippers into the bottles. Do not screw on the caps.
9. Place collection trays below the reagent sippers in the dNTP positions. Press **Next** to begin cleaning.
10. When cleaning is complete, remove the bottles and sippers from the W1, W2 and W3 positions. Leave the reagent sippers and collection trays in place. Press **Next** to return to the main menu and proceed to initialization.

## Chlorite cleaning

**Note:** Prepare a stock of 1 M NaOH each week by diluting 10 M NaOH with 18 MΩ water.

1. Empty any remaining solution from each cleaning bottle (two 250-mL bottles and one 2-L bottle) and rinse each bottle twice with ~100 mL of 18 MΩ water.
2. Fill a glass bottle with 1 L of 18 MΩ water and add an Ion PGM™ Cleaning Tablet (chlorite tablet). Allow the tablet to completely dissolve (~10 minutes).
3. When the tablet has dissolved, add 1 mL of 1 M NaOH and filter the solution using a 0.22-μm or 0.45-μm filter. Use the chlorite solution within 2–3 hours. Discard any unused solution after this time.
4. Press **Clean** on the touchscreen, and select the **Chlorite cleaning** checkbox. Press **Next**.
5. Using ungloved hands, secure a used chip designated for cleaning in the chip clamp.

---

**IMPORTANT!** Always make sure that both red rubber gasket port fittings are securely in place when securing chips with the chip clamp. Failure to do so can result in a spill hazard and instrument damage.

---

6. Remove all wash and reagent bottles attached to the instrument. Keep the sippers in place at all positions. Press **Next**.
7. Add 250 mL of the filtered chlorite solution to an empty 250-mL cleaning bottle.
8. Rinse the outside of the sipper tube in the W1 position on the instrument with a squirt bottle containing 18 MΩ water.
9. Attach the 250-mL bottle with the filtered chlorite solution to the W1 position. Make sure that the W1 cap is tight. Press **Next**.
10. Place the empty 2-L cleaning bottle in the W2 position and the empty 250-mL bottle in the W3 position, and insert the sippers into the bottles. Do not screw on the caps.

11. Place collection trays below the reagent sippers in the dNTP positions. Press **Next** to begin cleaning.
12. When prompted, remove the bottle containing the chlorite solution from the W1 position.
13. Rinse the outside of the W1 sipper tube with a squirt bottle containing 18 MΩ water.
14. Fill a clean 250-mL bottle with 250 mL of 18 MΩ water and attach the bottle in the W1 position. Make sure the cap is tight. Press **Next** to begin the water rinse.
15. When cleaning is complete, remove the bottles and sippers from the W1, W2 and W3 positions. Leave the reagent sippers and collection trays in place. Press **Next** to return to the main menu and proceed to initialization.

## Initialize the Ion PGM™ System

Initialization takes ~1 hour. As part of the initialization process, first prepare the Wash and Reagent Bottles as described in this section.

### Materials required

#### Materials provided in the kit

- Ion PGM™ Hi-Q™ Sequencing dGTP
- Ion PGM™ Hi-Q™ Sequencing dCTP
- Ion PGM™ Hi-Q™ Sequencing dATP
- Ion PGM™ Hi-Q™ Sequencing dTTP
- Ion PGM™ Hi-Q™ Sequencing W2 Solution (stored protected from light)
- Ion PGM™ Sequencing W3 Solution
- Wash 1 and Wash 3 Bottles and sipper tubes
- Wash 2 Bottle and sipper tubes (bottle must be conditioned prior to first use, as described in “Condition the Wash 2 Bottle for first use” on page 22)
- Wash 2 Bottle Conditioning Solution
- Reagent Bottles and sipper tubes

#### Other materials and equipment

- Used chip (leave chip on the instrument during initialization)
- 18 MΩ water
- 100 mM NaOH (prepared daily)
- Ice
- 5-mL and 25-mL pipettes
- Filtered and unfiltered pipette tips and pipettes
- Vortex mixer
- Microcentrifuge
- **Optional:** Ion PGM™ Sequencing Sippers Kit (Cat. no. 4478682)

## Initialization guidelines

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**IMPORTANT!** Handle nucleotides carefully to avoid cross-contamination. Always change gloves after removing used sipper tubes from the Ion PGM™ System to avoid cross contamination of the nucleotides. Also change gloves after handling concentrated dNTP stocks.

---

- For each initialization, the first run should be started within 1 hour after initialization, and the last run must be started within 27 hours after initialization.
- Make sure that you have updated the Ion PGM™ Torrent Suite™ System and Ion PGM™ System software to Version 4.2 or later.

### Bottle usage

- Wash 2 Bottles may be used for up to 40 initializations, after which you can use them in the cleaning procedure.
- Wash 1 and 3 Bottles may be used for up to four initializations, after which you can reuse them in the cleaning procedure.
- Replace the Reagent Bottles and sipper tubes every time you initialize.

## Before initialization

1. Remove the dNTP stock solutions from the freezer and begin thawing on ice.
2. Check the tank pressure for the nitrogen gas. When the tank pressure drops below 500 psi, change the tank (see also “Gas cylinders” on page 17).

## Prepare the Wash 2 Bottle

### Note:

- Do not remove the old sippers from the dNTP ports until instructed to do so.
- Load the bottles as quickly as possible to prevent atmospheric CO<sub>2</sub> from reducing the pH of the Wash 2 solution.
- For all the following steps, pour the 18 MΩ water directly from the purification system into the Wash 2 Bottle. Do not use water that has been collected or measured in any other containers.

---

**IMPORTANT!** Do not let the new sippers touch any surfaces.

---

1. Rinse the Wash 2 Bottle (2 L) three times with 200 mL of 18 MΩ water.
2. Prepare 500 µL of 100 mM NaOH by diluting 50 µL of 1 M NaOH in 450 µL of nuclease-free water.

3. If your 18 MΩ water system has a spigot, extend it into **but not below** the neck of the Wash 2 Bottle. Otherwise, position the nozzle as close to the mouth of the bottle as possible.



**Note:** If your water system has a digital display, make sure it reads "18 MΩ" throughout filling the bottle. If not, see Appendix A, "Troubleshooting".

4. Fill the bottle to the mold line with 18 MΩ water. The volume of water will be ~2 liters. (You can mark the mold line on the bottle for clarity, as shown in the image above.)

**Note:** If you are preparing bottles for multiple sequencers, cap each bottle immediately after filling, and leave capped until you are ready to add Ion PGM™ Hi-Q™ Sequencing W2 Solution.

5. Add the entire bottle of Ion PGM™ Hi-Q™ Sequencing W2 Solution to the Wash 2 Bottle.



**Note:** Keep the Ion PGM™ Hi-Q™ Sequencing W2 Solution bottle to scan the barcode during the initialization procedure.

- Using a P200 pipette, add 70 µL of 100 mM NaOH to the Wash 2 Bottle.

**Note:** Different sites may require adding different volumes of 100 mM NaOH. Some sites, for example, may require doubling the volume to 140 µL. See “Error message: Added too much W1 to W2” on page 63 for information on determining the volume of 100 mM NaOH to add.

- Cap the bottle and invert five times to mix, and immediately proceed through the rest of the initialization procedure.

---

**IMPORTANT!** Do not store the mixed Wash 2 Bottle.

---

## Prepare the Wash 1 and Wash 3 Bottles

**Note:** For the following steps, label the Wash 1 and Wash 3 Bottles to avoid confusion.

- Rinse the Wash 1 and Wash 3 Bottles three times with 50 mL of 18 MΩ water.
- Wash 1 Bottle:** Add 350 µL of freshly prepared 100 mM NaOH to the Wash 1 Bottle and cap the bottle.
- Wash 3 Bottle:** Add Ion PGM™ Sequencing W3 Solution to the 50-mL line marked on the Wash 3 Bottle and cap the bottle.

## Begin the initialization

**Note:**

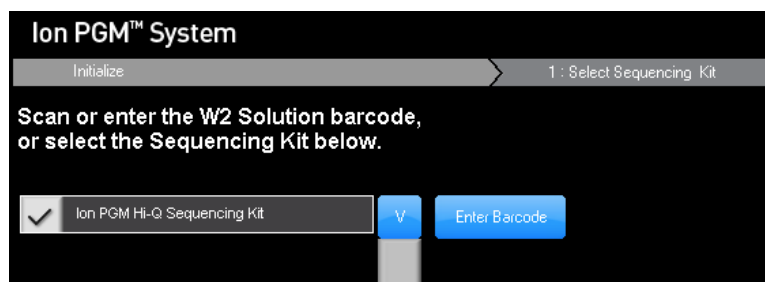
- Do not remove the old sipper tubes from the dNTP ports until instructed to do so.
- Load the bottles as quickly as possible to prevent atmospheric CO<sub>2</sub> from reducing the pH of the Wash 2 Bottle solution.

---

**IMPORTANT!** Do not let the new sipper tubes touch any surfaces.

---

- On the main menu, press **Initialize**.
- In the next screen, click **Enter barcode** to scan or enter the barcode on the Ion PGM™ Hi-Q™ Sequencing W2 Solution bottle, or the 2D barcode on the Ion PGM™ Hi-Q™ Sequencing Solutions box. Alternatively, select **Ion PGM™ Hi-Q™ Sequencing Kit** from the dropdown list. Press **Next**.




---

**IMPORTANT!** Be careful to scan the correct barcode or select the correct kit type, to ensure proper pH adjustment.

---

3. The system will check the gas pressure. If the pressure is sufficient, confirm that the cleaning chip, reagent sipper tubes, and collection trays are in place, and press **Next** to begin the initialization. If the gas pressure is low, press **Yes** to re-check the pressure. If the pressure remains low, contact Technical Support.
4. Wearing clean gloves, firmly attach a new, long gray sipper to the cap in the W2 position. **Do not let the sipper touch any surfaces.**

---

**IMPORTANT!** Be careful to firmly attach sippers to the ports. Loosely attached sippers may adversely affect results.

---



5. Immediately attach the prepared Wash 2 Bottle in the W2 position and tighten the cap. Press **Next**.
6. Change gloves and firmly install new sipper tubes (short gray) in the caps in the W1 and W3 positions.
7. Immediately attach the prepared Wash 1 and 3 Bottles and tighten the caps. Press **Next**.
8. The sequencer will begin adjusting the pH of the W2 Solution, which takes ~30 minutes. After 15 minutes, check the instrument touchscreen to confirm that initialization is proceeding normally.

**Note:**

- If an error occurs during the automatic pH process, note the error message and proceed to “Initialization—Auto pH errors” on page 59.
- During the process, you can begin preparing the Reagent Bottles with dNTPs as described in the next section.

### Prepare the 50-mL Reagent Bottles with dNTPs

1. Use the labels provided with the kit to label four new Reagent Bottles as dGTP, dCTP, dATP, and dTTP.
2. Confirm that no ice crystals are visible in each thawed dNTP stock solution. Vortex each tube to mix, and centrifuge to collect the contents. Keep the dNTP stock solutions on ice throughout this procedure.

---

**IMPORTANT!** To avoid cross-contamination in the next step, open only one dNTP stock tube at a time and use a fresh pipette tip for each aliquot.

---

3. Using separate filtered pipette tips and clean gloves, carefully transfer 20 µL of each dNTP stock solution into its respective Reagent Bottle.
4. Cap each Reagent Bottle and store on ice until you are ready to attach it to the instrument. Place the remaining dNTP stocks back into –20°C for storage.

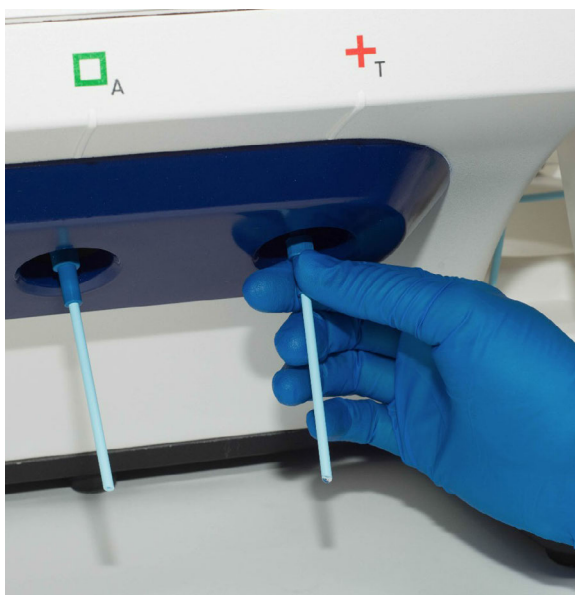
### Attach the sipper tubes and Reagent Bottles

1. After the wash solutions have initialized, follow the touchscreen prompts to remove the used sipper tubes and collection trays from the dNTP ports.
2. Change gloves, then firmly insert a new sipper tube (blue) into each dNTP port. Do not let the sipper touch any surfaces.

---

**IMPORTANT!** Be careful to firmly push each sipper onto the port. Loosely attached sippers may adversely affect results.

---



3. Attach each prepared Reagent Bottle to the correct dNTP port (e.g., the dGTP tube on the port marked "G," as shown below) and tighten firmly by hand until snug. Press **Next**.



**Note:** The instrument checks the pressure of the Reagent Bottles and Wash Bottles. If a bottle leaks, check that it is tightly attached to the instrument. If it continues to leak, replace it. If the instrument still does not pass the leak check, contact Technical Support.

4. Follow the touchscreen prompts to complete initialization. The instrument will fill each Reagent Bottle with 40 mL of W2 Solution.
5. At the end of initialization, Ion PGM™ System will measure the pH of the reagents:
  - If every reagent is in the target pH range, a green **Passed** screen will be displayed.
  - If a red failure screen appears, see Appendix A, "Troubleshooting".
6. Press **Next** to finish the initialization process and return to the main menu.
7. Proceed to the appropriate sequencing protocol for your chip type.

# Sequencing protocol—Ion 316™ Chips or Ion 318™ Chips

Use the following sequencing protocol with Ion 316™ Chips or Ion 318™ Chips. For the Ion 314™ Chips, see Chapter 6, “Sequencing protocol—Ion 314™ Chip v2”.

## Materials required

### Materials provided in the Ion PGM™ Hi-Q™ Sequencing Kit

- Ion PGM™ Hi-Q™ Sequencing Polymerase
- Sequencing Primer
- Control Ion Sphere™ Particles (ISPs)
- Annealing Buffer

### Materials provided in the Ion PGM™ Controls Kit v2

- (Optional) Ion PGM™ Ion Sphere™ Test Fragments

### Other materials and equipment

- Ion 316™ Chip v2 or Ion 318™ Chip v2
- Enriched template-positive ISPs
- 0.2-mL PCR tube (non-polystyrene)
- Rainin® SR-L200F pipette and tips
- Vortex mixer
- Ion PGM™ Chip Minifuge
- Thermal cycler with heated lid (programmed at 95°C for 2 minutes and 37°C for 2 minutes)
- Barcode scanner (included with the Ion PGM™ System)

## Before starting

- Thaw the Sequencing Primer on ice.
- Make sure that you have updated the Ion PGM™ Torrent Suite™ System and Ion PGM™ System software to Version 4.2 or later.

**Note:** For each initialization, the first run should be started within 1 hour after initialization, and the last run must be started within 27 hours after initialization.

---

**IMPORTANT!** The ISPs are difficult to see. To avoid aspirating the particles:

- When centrifuging the ISPs, orient the tab of the tube lid so that it is pointing away from the center of the centrifuge, to indicate where the pellet will be formed.
  - Always remove supernatant from the tube from the top down
- 

## Optional: Prepare Ion Sphere Test Fragments

If you are performing an installation or troubleshooting sequencing run:

1. Vortex the Ion PGM™ Ion Sphere™ Test Fragments from the Ion PGM™ Controls Kit v2 (Cat. no. 4482010) and pulse spin in a picofuge for 2 seconds before taking aliquots.
2. Add 5 µL of Ion PGM™ Ion Sphere™ Test Fragments to 100 µL of Annealing Buffer in a 0.2-mL non-polystyrene PCR tube.
3. Skip directly to annealing the sequencing primer.

## Add controls to the enriched, template-positive ISPs

1. Vortex the Control Ion Sphere™ Particles and pulse spin in a picofuge for 2 seconds before taking aliquots.
2. Add 5 µL of Control ISPs directly to the entire volume of enriched, template-positive ISPs (prepared using your template preparation method) in a 0.2-mL non-polystyrene PCR tube.
3. Proceed to annealing the sequencing primer.

## Anneal the Sequencing Primer

1. Mix the tube containing the ISPs (or test fragments) by thoroughly pipetting up and down.
2. Place the tube in a microcentrifuge with an appropriate tube adapter. Orient the tab of the tube lid so that it is pointing away from the center of the centrifuge, to indicate where the pellet will be formed.
3. Centrifuge for 2 minutes at 15,500 × g.
4. Keeping the pipette plunger depressed, insert a pipette tip into the tube containing the pelleted ISPs and carefully remove the supernatant from the top down, avoiding the side of the tube with the pellet (i.e., the side with the tab on the tube lid). Discard the supernatant. Leave ~15 µL in the tube (visually compare to 15 µL of liquid in a separate tube).

5. Ensure that the Sequencing Primer is completely thawed prior to use (no ice crystals should be visible).
6. Vortex the primer for 5 seconds, then pulse spin in a microfuge for 3–5 seconds to collect the contents. Leave on ice until ready to use.
7. Add 12 µL of Sequencing Primer to the ISPs, and confirm that the total volume is 27 µL (add Annealing Buffer if necessary).
8. Pipet the mixture up and down thoroughly to disrupt the pellet.

---

**IMPORTANT!** Make sure that the pipette tip is at the bottom of the tube during mixing to avoid introducing air bubbles into the sample.

---

9. Program a thermal cycler for 95°C for 2 minutes and then 37°C for 2 minutes, using the heated lid option.
10. Place the tube in the thermal cycler and run the program. After cycling, the reaction can remain in the cycler at room temperature (20–30°C) while you proceed with Chip Check.

## Perform Chip Check

Chip Check tests the chip and ensures that it is functioning properly prior to loading the sample.

---

### IMPORTANT!

- To avoid damage due to electrostatic discharge (ESD), **do not place the chip directly on the bench or any other surface.** Always place the chip either on the grounding plate on the Ion PGM™ Sequencer or in the Ion PGM™ Chip Minifuge adapter bucket.
  - To avoid ESD damage, **do not wear gloves** when transferring chips on and off the instrument.
- 

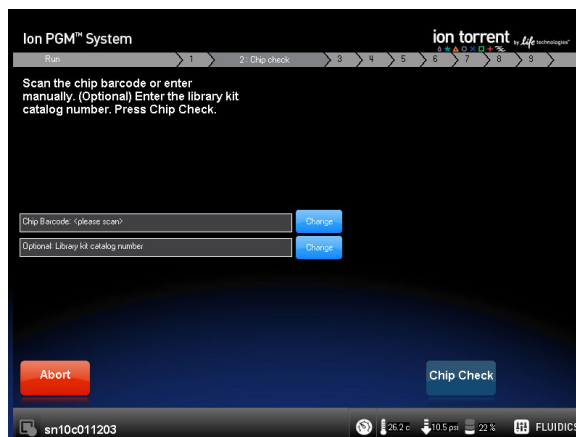
1. On the main menu of the Ion PGM™ Sequencer touchscreen, press **Run**. Remove the waste bottle and completely empty it. Press **Next**.
2. When prompted to insert a cleaning chip, use the same used chip that was used for initialization. Press **Next** to clean the fluid lines.
3. Remove gloves, and ground yourself by touching the grounding pad on the sequencer. Remove a new chip from its packaging and label it to identify the experiment (save the chip package). Press **Next**.

4. Replace the old chip in the chip socket with the new one for the experiment. Close the chip clamp, then press **Next**.



5. When prompted, use the scanner to scan the barcode located on the chip package, or press **Change** to enter the barcode manually. Optionally, you can also enter the library kit catalog number.

**Note:** A chip cannot be run without scanning or entering the barcode.



6. Press **Chip Check**. During the initial part of Chip Check, visually inspect the chip in the clamp for leaks.

**Note:**

- If there is a leak, press the **Abort** button immediately to stop the flow to the chip. Proceed to Appendix A, “Troubleshooting”.
- **The chip socket can be damaged by rubbing or wiping its surface.** Never rub or wipe the socket to clean up leaks. See Appendix A, “Troubleshooting” for more information.

7. When Chip Check is complete:
  - If the chip passes, press **Next**.
  - If the chip fails, open the chip clamp, re-seat the chip in the socket, close the clamp, and press **Calibrate** to repeat the procedure. If the chip passes, press **Next**. If the chip still fails, press **Main Menu** and restart the experiment with a new chip. See Appendix A, “Troubleshooting” for more information.

**Note:** To return *damaged* chips, contact Technical Support.
8. Following a successful Chip Check, completely empty the waste bottle and select the **Waste bottle is empty** checkbox on the touchscreen. Press **Next**.

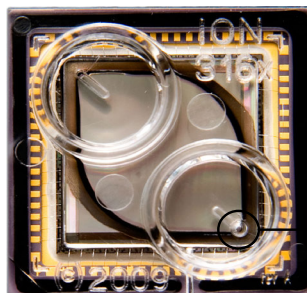
## Bind the Sequencing Polymerase to the ISPs

1. Remove the Ion PGM™ Hi-Q™ Sequencing Polymerase from storage and flick mix with your finger tip four times. Pulse spin for 3–5 seconds. Place on ice.
2. After annealing the Sequencing Primer, remove the ISPs from the thermal cycler and add 3 µL of Ion PGM™ Hi-Q™ Sequencing Polymerase to the ISPs, for a total final volume of 30 µL.
3. Pipet the sample up and down to mix, and incubate at room temperature for 5 minutes.

## Prepare and load the chip

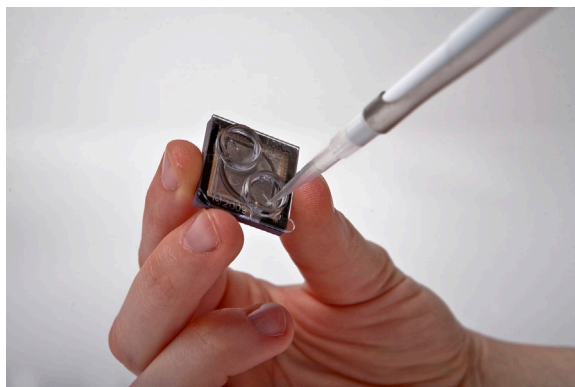
### Remove liquid from the chip

Ion 316™/  
Ion 318™ Chip v2



Loading port

1. Following Chip Check, remove the new chip from the Ion PGM™ Sequencer. Insert a used chip in the chip clamp while loading the new chip.
2. Tilt the new chip 45 degrees so that the loading port is the lower port, as shown below.



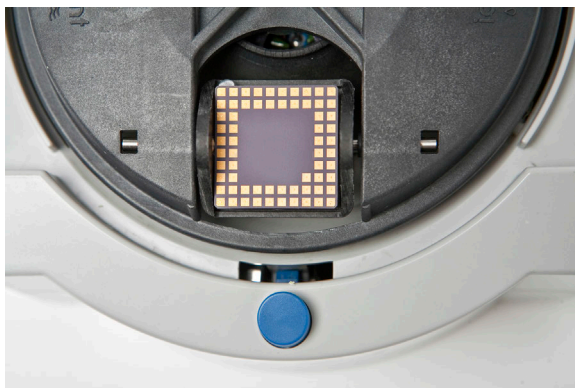
3. Insert the pipette tip firmly into the loading port and remove as much liquid as possible from the loading port. Discard the liquid.

---

**IMPORTANT!** For the next steps, if you are preparing one chip at a time, balance the chip in the Ion PGM™ Chip Minifuge rotor with a used chip of the same chip type and orientation. Be careful to balance an upside-down chip with another upside-down chip. Mark the used chip with a laboratory marker to differentiate it from the new chip.

---

4. Place the chip **upside-down** in the minifuge bucket and transfer the bucket to the **with the chip tab pointing in** (toward the center of the minifuge), as shown below. Balance the bucket with another chip.



5. Centrifuge for 5 seconds to completely empty the chip.



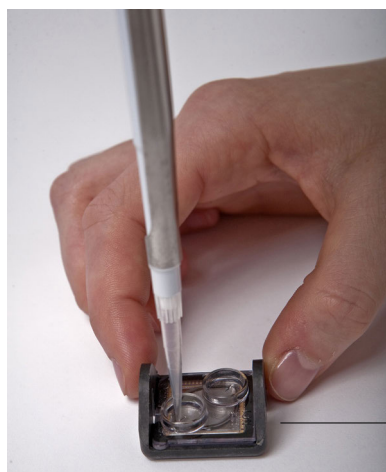
**CAUTION!** Allow the minifuge to come to a complete stop before opening the lid.

---

6. Remove the chip from the bucket and wipe the bucket with a disposable wipe to remove any liquid. Place the chip right-side up in the bucket.

## Load the chip

**Note:** When loading liquid into the chip, keep the pipette tip at a 90° angle to the chip, press the tip firmly into the circular loading port, and apply gentle pressure between the pipette tip and chip.



Centrifuge  
adapter bucket

1. Place the chip in the bucket on a firm, flat surface. Following polymerase incubation, collect the entire sample (~30 µL) into a Rainin® SR-L200F pipette tip and insert the tip firmly into the loading port of the chip.
2. With the pipette unlocked, apply gentle pressure between the tip and chip and slowly dial down the pipette (~1 µL per second) to deposit the ISPs. To avoid introducing bubbles into the chip, leave a small amount of sample in the pipette tip (~0.5 µL).



**Note:** Do not remove the pipette tip from the port during the dial-down process, since this can introduce air bubbles and inhibit loading.

3. Remove and discard any displaced liquid from the other port of the chip.

4. Transfer the chip in the bucket to the minifuge **right-side up with the chip tab pointing in** (toward the center of the minifuge).



5. Centrifuge for 30 seconds.
6. Remove the bucket from the minifuge and place it on a flat surface. Mix the sample in the chip as follows:
- Set the pipette volume to 30  $\mu$ L.
  - Tilt the chip 45 degrees so that the loading port is the lower port, and insert the pipette tip into the loading port.
  - Without removing the tip, slowly pipet the sample in and out of the chip three times. **Pipet slowly to avoid creating bubbles.**
7. Return the bucket to the minifuge **with the chip tab pointing out** (away from the center of the minifuge). Centrifuge for 30 seconds.



8. Repeat the chip mixing in step 6, then spin for 30 seconds **with the chip tab pointing in** (toward the center of the minifuge).
9. **Template prepared with the Ion PGM™ Template OT2 400 Kit only:** Repeat the chip mixing step one more time, except pipet the mixture in and out of the chip *five* times.
10. Tilt the chip at a 45-degree angle and slowly remove as much liquid as possible from the loading port by dialing the pipette. Discard the liquid.

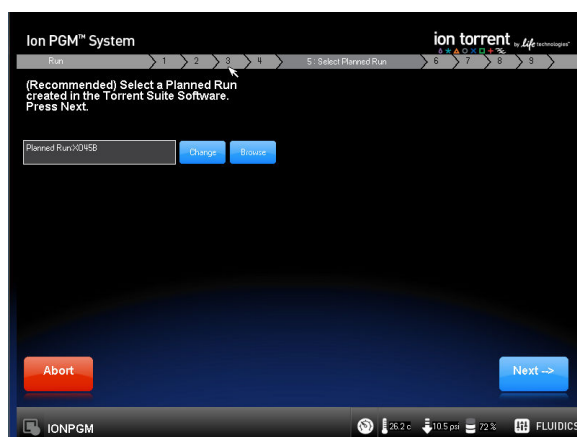
11. If some liquid remains in the chip, perform a 5-second quick spin with the chip tab pointing out and remove and discard any additional liquid. **Do not spin the chip upside down.**
12. If some liquid remains in the chip after the quick spin, lightly and rapidly tap the point of the chip tab against the benchtop a few times, and remove and discard any collected liquid. Do not flush the chip.
13. When chip loading is complete, press **Next** on the touchscreen and proceed immediately to performing the run.

## Select the Planned Run and perform the run

### Select the Planned Run

1. Press **Browse** next to the **Planned Run** field and select the name of the plan you created, then touch **Next**.

**Note:** The Ion PGM™ Sequencer automatically populates this field for barcoded Ion chips.



2. Confirm that the settings are correct. If necessary, make any changes using the touchscreen controls.



**Note:** If the number of flows (cycles) to be run cannot be selected, there may not be enough disk space to store the experiment data. Touch **Data Mngt** to start the Data Management application (this can also be accessed from the Tools Menu) and delete old runs from the Ion PGM™ System.

## Perform the run

1. After you enter the Planned Run, press **Next** to verify the experimental setup. Press **OK** to confirm the settings or press **Cancel** to return to the touchscreen to adjust the settings.
2. When prompted by the instrument, load and clamp the chip, then press **Next**.
3. At the beginning of the run, visually inspect the chip in the clamp for leaks before closing the cover. The instrument will flush any loose ISPs from the chip and begin calibrating the chip.
4. When the calibration is complete (~1 minute), the touchscreen will indicate whether calibration was successful.
  - If the chip passes calibration, press **Next** to proceed with the sequencing run.
  - If the chip fails calibration, see “Error message: Calibration FAILED” on page 58.
5. After 60 seconds, the run will automatically begin, or press **Next** to begin the run immediately.

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**IMPORTANT!** During a run, avoid touching the instrument and any of the attached bottles or tubes, as this may reduce the quality of the measurements.

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6. When the run is complete, leave the chip in place, then touch **Next** to return to the Main Menu. You can then remove the chip and proceed with another run or perform a cleaning/initializing if required.

**Note:** See “Cleaning schedule” on page 23 to determine whether cleaning is required after the run.



# Sequencing protocol—Ion 314<sup>TM</sup> Chip v2

Use the following sequencing protocol with the Ion 314<sup>TM</sup> Chip v2. For the Ion 316<sup>TM</sup> Chip v2 or Ion 318<sup>TM</sup> Chip v2, see Chapter 5, “Sequencing protocol—Ion 316<sup>TM</sup> Chips or Ion 318<sup>TM</sup> Chips”.

## Materials required

### Materials provided in the Ion PGM<sup>TM</sup> Hi-Q<sup>TM</sup> Sequencing Kit

- Ion PGM<sup>TM</sup> Hi-Q<sup>TM</sup> Sequencing Polymerase
- Sequencing Primer
- Control Ion Sphere<sup>TM</sup> Particles
- Annealing Buffer

### Materials provided in the Ion PGM<sup>TM</sup> Controls Kit v2

- (Optional) Ion PGM<sup>TM</sup> Ion Sphere<sup>TM</sup> Test Fragments

### Other materials and equipment

- Ion 314<sup>TM</sup> Chip v2
- Enriched template-positive ISPs
- 0.2-mL PCR tube (non-polystyrene)
- Rainin<sup>®</sup> SR-L200F pipette and tips
- Vortex mixer
- Ion PGM<sup>TM</sup> Chip Minifuge
- Thermal cycler with heated lid (programmed at 95°C for 2 minutes and 37°C for 2 minutes)
- Barcode scanner (included with the Ion PGM<sup>TM</sup> System)

## Before starting

- Thaw the Sequencing Primer on ice.
- Make sure that you have updated the Ion PGM<sup>TM</sup> Torrent Suite<sup>TM</sup> System and Ion PGM<sup>TM</sup> System software to Version 4.2 or later.

**Note:** For each initialization, the first run should be started within 1 hour after initialization, and the last run must be started within 27 hours after initialization.

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**IMPORTANT!** The ISPs are difficult to see. To avoid aspirating the particles:

- When centrifuging the ISPs, orient the tab of the tube lid so that it is pointing away from the center of the centrifuge, to indicate where the pellet will be formed.
  - Always remove supernatant from the tube from the top down
- 

## Optional: Prepare Ion Sphere Test Fragments

If you are performing an installation or troubleshooting sequencing run:

1. Vortex the Ion PGM™ Ion Sphere™ Test Fragments from the Ion PGM™ Controls Kit v2 (Cat. no. 4482010) and pulse spin in a microfuge for 2 seconds before taking aliquots.
2. Add 5 µL of Ion PGM™ Ion Sphere™ Test Fragments to 100 µL of Annealing Buffer in a 0.2-mL non-polystyrene PCR tube.
3. Skip directly to annealing the sequencing primer.

## Add controls to the enriched, template-positive ISPs

**Note:** The Ion 314™ Chip uses only half the volume of enriched, template positive ISPs prepared using the template kit. If you are performing an installation or troubleshooting sequencing run:

1. Transfer **half the volume** of enriched, template-positive ISPs to a new 0.2-mL non-polystyrene PCR tube and store at 2–8°C for up to 1 week. They may be used for another sequencing run.
2. Vortex the Control Ion Sphere™ Particles and pulse spin in a microfuge for 2 seconds before taking aliquots.
3. Add 5 µL of Control Ion Sphere™ Particles directly to the entire volume of enriched, template-positive ISPs in a 0.2-mL non-polystyrene PCR tube.
4. Proceed to annealing the sequencing primer.

## Anneal the sequencing primer

1. Mix the tube containing the ISPs (or test fragments) by thoroughly pipetting up and down.
  2. Place the tube in a microcentrifuge with an appropriate tube adapter. Orient the tab of the tube lid so that it is pointing away from the center of the centrifuge, to indicate where the pellet will be formed.
  3. Centrifuge for 2 minutes at  $15,500 \times g$ .
  4. Keeping the pipette plunger depressed, insert a pipette tip into the tube containing the pelleted ISPs and carefully remove the supernatant from the top down, avoiding the side of the tube with the pellet (i.e., the side with the tab on the tube lid). Discard the supernatant. Leave  $\sim 3 \mu\text{L}$  in the tube (visually compare to  $3 \mu\text{L}$  of liquid in a separate tube).
  5. Ensure that the Sequencing Primer is completely thawed prior to use (no ice crystals should be visible).
  6. Vortex the primer for 5 seconds, then pulse spin in a microfuge for 3–5 seconds to collect the contents. Leave on ice until ready to use.
  7. Add  $3 \mu\text{L}$  of Sequencing Primer to the ISPs, and confirm that the total volume is  $6 \mu\text{L}$  (add Annealing Buffer if necessary).
  8. Pipet the mixture up and down thoroughly to disrupt the pellet.
- 
- IMPORTANT!** Make sure that the pipette tip is at the bottom of the tube during mixing to avoid introducing air bubbles into the sample.
- 
9. Program a thermal cycler for  $95^{\circ}\text{C}$  for 2 minutes and then  $37^{\circ}\text{C}$  for 2 minutes, using the heated lid option.
  10. Place the tube in the thermal cycler and run the program. After cycling, the reaction can remain in the cycler at room temperature ( $20\text{--}30^{\circ}\text{C}$ ) while you proceed with Chip Check.

## Perform Chip Check

Chip Check tests the chip and ensures that it is functioning properly prior to loading the sample.

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### IMPORTANT!

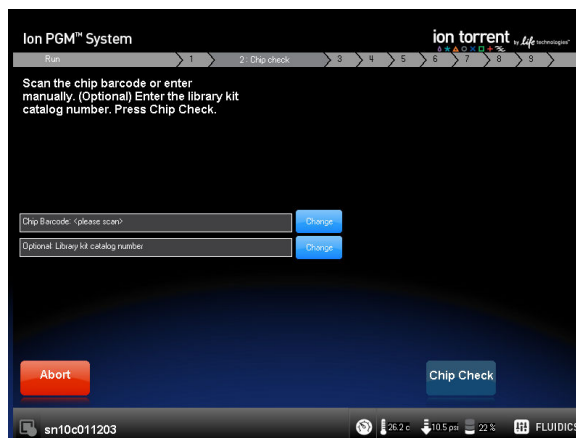
- To avoid damage due to electrostatic discharge (ESD), **do not place the chip directly on the bench or any other surface**. Always place the chip either on the grounding plate on the Ion PGM™ Sequencer or in the Ion PGM™ Chip Minifuge adapter bucket.
  - To avoid ESD damage, **do not wear gloves** when transferring chips on and off the instrument.
- 

1. On the main menu of the Ion PGM™ Sequencer touchscreen, press **Run**. Remove the waste bottle and completely empty it. Press **Next**.
2. When prompted to insert a cleaning chip, use the same used chip that was used for initialization. Press **Next** to clean the fluid lines.
3. Remove gloves, and ground yourself by touching the grounding pad on the sequencer. Remove a new chip from its packaging and label it to identify the experiment (save the chip package). Press **Next**.
4. Replace the old chip in the chip socket with the new one for the experiment. Close the chip clamp, then press **Next**.



5. When prompted, use the scanner to scan the barcode located on the chip package, or press **Change** to enter the barcode manually. Optionally, you can also enter the library kit catalog number.

**Note:** A chip cannot be run without scanning or entering the barcode.



6. Press **Chip Check**. During the initial part of Chip Check, visually inspect the chip in the clamp for leaks.

**Note:**

- If there is a leak, press the **Abort** button immediately to stop the flow to the chip. Proceed to Appendix A, “Troubleshooting”.
- **The chip socket can be damaged by rubbing or wiping its surface.** Never rub or wipe the socket to clean up leaks. See Appendix A, “Troubleshooting” for more information.

7. When Chip Check is complete:

- If the chip passes, press **Next**.
- If the chip fails, open the chip clamp, re-seat the chip in the socket, close the clamp, and press **Calibrate** to repeat the procedure. If the chip passes, press **Next**. If the chip still fails, press **Main Menu** and restart the experiment with a new chip. See Appendix A, “Troubleshooting” for more information.

**Note:** To return *damaged* chips, contact Technical Support.

8. Following a successful Chip Check, completely empty the waste bottle and select the **Waste bottle is empty** checkbox on the touchscreen. Press **Next**.

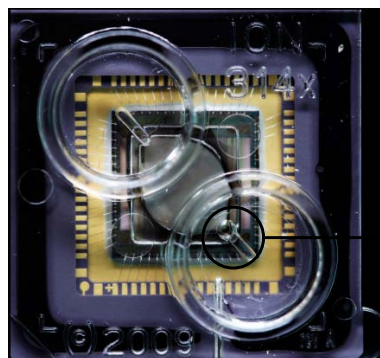
## Bind the Sequencing Polymerase to the ISPs

1. Remove the Ion PGM™ Hi-Q™ Sequencing Polymerase from storage and flick mix with your finger tip four times. Pulse spin for 3–5 seconds. Place on ice.
2. After annealing the Sequencing Primer, remove the ISPs from the thermal cycler and add 1 µL of Ion PGM™ Hi-Q™ Sequencing Polymerase to the ISPs, for a total final volume of 7 µL.
3. Pipet the sample up and down to mix, and incubate at room temperature for 5 minutes.

## Prepare and load the chip

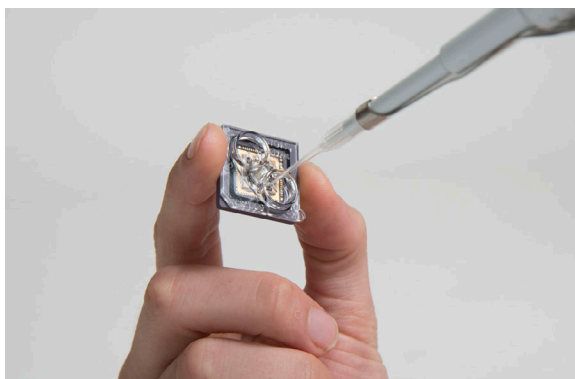
Remove liquid  
from the chip

Ion 314™ Chip v2



Loading port

1. Following Chip Check, remove the new chip from the Ion PGM™ Sequencer. Insert a used chip in the chip clamp while loading the new chip.
2. Tilt the new chip 45 degrees so that the loading port is the lower port, as shown below.



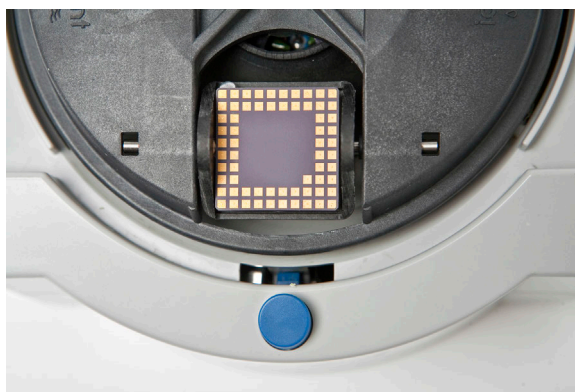
3. Insert the pipette tip firmly into the loading port and remove as much liquid as possible from the loading port. Discard the liquid.

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**IMPORTANT!** For the next steps, if you are preparing one chip at a time, balance the chip in the Ion PGM™ Chip Minifuge rotor with a used chip of the same chip type and orientation. Be careful to balance an upside-down chip with another upside-down chip. Mark the used chip with a laboratory marker to differentiate it from the new chip.

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4. Place the chip **upside-down** in the minifuge bucket and transfer the bucket to the **with the chip tab pointing in** (toward the center of the minifuge), as shown below. Balance the bucket with another chip.



5. Centrifuge for 5 seconds to completely empty the chip.



**CAUTION!** Allow the minifuge to come to a complete stop before opening the lid.

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6. Remove the chip from the bucket and wipe the bucket with a disposable wipe to remove any liquid. Place the chip right-side up in the bucket.

## Load the chip

**Note:** When loading liquid into the chip, keep the pipette tip at a 90° angle to the chip, press the tip firmly into the circular loading port, and apply gentle pressure between the pipette tip and chip.



1. Place the chip in the bucket on a firm, flat surface. Following polymerase incubation, collect the entire sample (~7  $\mu\text{L}$ ) into a Rainin® SR-L200F pipette tip and insert the tip firmly into the loading port of the chip.
2. With the pipette unlocked, apply gentle pressure between the tip and chip and slowly dial down the pipette (~1  $\mu\text{L}$  per second) to deposit the ISPs. To avoid introducing bubbles into the chip, leave a small amount of sample in the pipette tip (~0.5  $\mu\text{L}$ ).



**Note:** Do not remove the pipette tip from the port during the dial-down process, since this can introduce air bubbles and inhibit loading.

3. Remove and discard any displaced liquid from the other port of the chip.
4. Transfer the chip in the bucket to the minifuge **right-side up with the chip tab pointing in** (toward the center of the minifuge).



5. Centrifuge for 30 seconds, then remove the chip from the centrifuge bucket.
6. Remove the bucket from the minifuge and place it on a flat surface. Mix the sample in the chip as follows:
  - a. Set the pipette volume to 5  $\mu$ L.
  - b. Tilt the chip 45 degrees so that the loading port is the lower port, and insert the pipette tip into the loading port.
  - c. Without removing the tip, slowly pipet the sample in and out of the chip three times. **Pipet slowly to avoid creating bubbles.**
7. Return the bucket to the minifuge **with the chip tab pointing out** (away from the center of the minifuge). Centrifuge for 30 seconds.



8. Repeat the chip mixing in step 6, then spin for 30 seconds **with the chip tab pointing in** (toward the center of the minifuge).

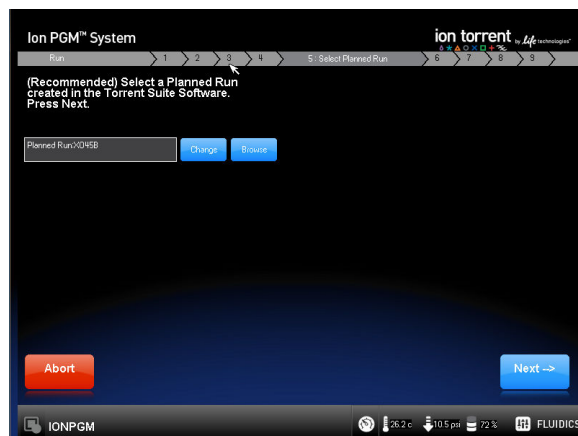
9. **Template prepared with the Ion PGM™ Template OT2 400 Kit only:** Repeat the chip mixing step one more time, except pipet the mixture in and out of the chip *five* times.
10. Tilt the chip at a 45-degree angle and slowly remove as much liquid as possible from the loading port by dialing the pipette. Discard the liquid.
11. If some liquid remains in the chip, perform a 5-second quick spin with the chip tab pointing out and remove and discard any additional liquid. **Do not spin the chip upside down.**
12. If some liquid remains in the chip after the quick spin, lightly and rapidly tap the point of the chip tab against the benchtop a few times, and remove and discard any collected liquid. Do not flush the chip.
13. When chip loading is complete, press **Next** on the touchscreen and proceed immediately to performing the run.

## Select the Planned Run and perform the run

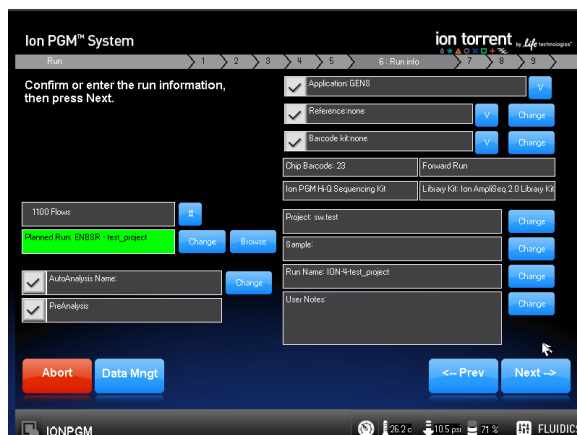
### Select the Planned Run

1. Press **Browse** next to the **Planned Run** field and select the name of the plan you created, then touch **Next**.

**Note:** The Ion PGM™ Sequencer automatically populates this field for barcoded Ion chips.



2. Confirm that the settings are correct. If necessary, make any changes using the touchscreen controls.



**Note:** If the number of flows (cycles) to be run cannot be selected, there may not be enough disk space to store the experiment data. Touch **Data Mngt** to start the Data Management application (this can also be accessed from the Tools Menu) and delete old runs from the Ion PGM™ System.

## Perform the run

1. After you enter the Planned Run, press **Next** to verify the experimental setup. Press **OK** to confirm the settings or press **Cancel** to return to the touchscreen to adjust the settings.
2. When prompted by the instrument, load and clamp the chip, then press **Next**.
3. At the beginning of the run, visually inspect the chip in the clamp for leaks before closing the cover. The instrument will flush any loose ISPs from the chip and begin calibrating the chip.
4. When the calibration is complete (~1 minute), the touchscreen will indicate whether calibration was successful.
  - If the chip passes calibration, press **Next** to proceed with the sequencing run.
  - If the chip fails calibration, see “Error message: Calibration FAILED” on page 58.
5. After 60 seconds, the run will automatically begin, or press **Next** to begin the run immediately.

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**IMPORTANT!** During a run, avoid touching the instrument and any of the attached bottles or tubes, as this may reduce the quality of the measurements.

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6. When the run is complete, leave the chip in place, then touch **Next** to return to the Main Menu. You can then remove the chip and proceed with another run or perform a cleaning/initializing if required.

**Note:** See “Cleaning schedule” on page 23 to determine whether cleaning is required after the run.

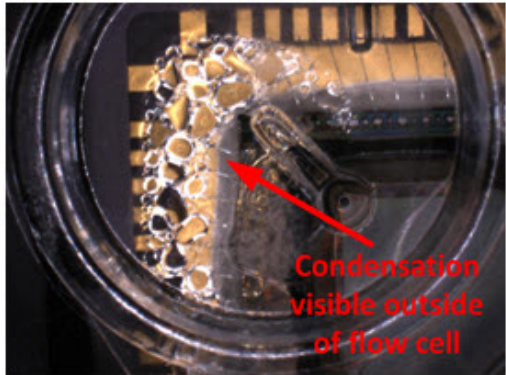


# Troubleshooting

## Chip Check

Observation	Possible cause	Recommended action
Chip Check fails	<ul style="list-style-type: none"> <li>Clamp not closed</li> <li>Chip not properly seated</li> <li>Debris on the chip socket</li> <li>Chip damaged</li> </ul>	<ol style="list-style-type: none"> <li>Open the chip clamp, remove the chip, and look for signs of water outside the flow cell: <div data-bbox="924 753 1421 1127" data-label="Image"> </div> </li> <li>If the chip appears damaged, replace it with a new one.</li> <li>Look for debris on the chip socket. Remove any debris by rinsing with 18 MΩ water and gently dabbing the socket with a lab wipe tissue. <p><b>IMPORTANT!</b> Never rub or wipe the socket. Rubbing the socket can damage it and cause it to fail.</p> </li> <li>Close the clamp and repeat the Chip Check.</li> <li>If the chip passes, click <b>Next</b>. If the chip fails, replace it with a new chip, scan the new chip's barcode, then press <b>Chip Check</b>.</li> <li>If Chip Check continues to fail, there could be a problem with the chip socket. Contact Technical Support.</li> </ol>

## Chip calibration (before loading sample)

Observation	Possible cause	Recommended action
Leak of unknown origin	<ul style="list-style-type: none"> <li>Chip leak</li> <li>Chip clamp not closed properly</li> <li>Problem with the chip clamp or socket</li> </ul>	<ol style="list-style-type: none"> <li>Press <b>Main Menu</b>.</li> <li>Open the chip clamp, remove the chip, and gently dab the chip socket with a lab wipe tissue to absorb any fluid. <b>IMPORTANT!</b> Never rub or wipe the socket. Rubbing the socket can damage it and cause it to fail.</li> <li>Rinse the socket with 18 MΩ water and gently absorb most of the water with the lab wipe.</li> <li>Repeat the rinse, then gently dab the chip socket until dry.</li> <li>Place a lab wipe on the grounding plate and dampen it with 18 MΩ water. Wipe the bottom of the chip on this wipe to remove salts from the chip contacts.</li> <li>Remove the wipe, dry the grounding plate, and place chip on grounding plate. Confirm that there is no condensation outside the flow cell:</li> </ol>  <ol style="list-style-type: none"> <li>Replace the chip with a new (unused) one if needed. <b>Note:</b> The new chip can be used for sequencing after initialization completes.</li> <li>Press <b>Run</b> to restart the experiment.</li> <li>When prompted to install the new chip, make certain that the chip clamp is fully closed.</li> <li>If the chip leaks again, clean the chip socket as described above. Continued leaking, even with new chips, may indicate a chip clamp or socket problem. Contact Technical support.</li> </ol>



Observation	Possible cause	Recommended action
Error message: Calibration FAILED	<ul style="list-style-type: none"><li>• Chip not seated in socket correctly</li><li>• Chip is damaged</li></ul>	<ol style="list-style-type: none"><li>1. Remove the chip and confirm that there is no leakage or debris on the chip socket. If leaking or debris is seen, follow the procedure for inspecting the chip and clearing debris as described under "Chip Check fails" and/or "Leak of unknown origin" above. If no leaking or debris is seen, reseal the chip in the socket.</li><li>2. Press <b>Calibrate</b> to repeat the calibration.</li><li>3. If the chip passes, press <b>Next</b>. If the chip still fails return to the main menu and restart the experiment with a new chip.</li><li>4. If you continue to have chip calibration issues, there may be an issue with the chip socket. Contact Technical Support.</li></ol>

## Chip calibration (after loading sample)

Observation	Possible cause	Recommended action
Leak of unknown origin	<ul style="list-style-type: none"> <li>Chip leak</li> <li>Chip clamp not closed properly</li> </ul>	<ol style="list-style-type: none"> <li>Press the <b>Abort</b> button.</li> <li>Open the chip clamp, remove the chip, and gently dab the chip socket with a lab wipe tissue to absorb any fluid. Do not rub or wipe the chip socket.</li> <li>Rinse the socket with 18 MΩ water and gently absorb most of the water with the lab wipe tissue.</li> <li>Repeat the rinse, then gently dab the chip socket until dry.</li> <li>Place a lab wipe tissue on the grounding plate and dampen it with 18 MΩ water. Wipe the bottom of the chip on this wipe to remove salts from the chip contacts.</li> <li>Remove the wipe, dry the grounding plate, and place the chip on the grounding plate. Check for condensation outside the flow cell: <div data-bbox="971 928 1474 1304" data-label="Image"> </div> </li> <li>If there is condensation or fluid, the chip is damaged and cannot be run.</li> <li>If there is no condensation or fluid, press <b>Calibrate</b> to restart the calibration procedure.</li> <li>If calibration passes and no leaks are visible, press <b>Next</b> to begin the experiment.</li> <li>If the chip leaks again, clean the chip and chip socket as described above. Continued leaking may indicate a chip clamp or socket problem. Contact Technical Support.</li> </ol>




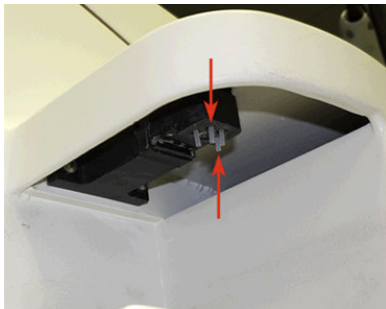
Observation	Possible cause	Recommended action
Error message: Calibration FAILED	<ul style="list-style-type: none"><li>• Chip not seated in socket correctly</li><li>• Chip is damaged</li></ul>	<ol style="list-style-type: none"><li>1. Remove the chip and check for leaks and/or debris on the chip socket, following the procedures described in "Chip Check fails" and/or "Leak of unknown origin," above. If no leaks or debris are visible, reseal the chip in the socket.</li><li>2. Press <b>Calibrate</b>.</li><li>3. If the chip passes, press <b>Next</b> to start the experiment. If the chip still fails, you can try resealing the chip multiple times and pressing <b>Calibrate</b>. If you are still unable to pass calibration, press <b>Next</b> to start the run anyhow—you may still get some data on your sample.</li><li>4. If you continue to have chip calibration issues, there may be an issue with the chip or chip socket. Contact Technical Support.</li></ol>

## Initialization—General errors

Observation	Possible cause	Recommended action
Error message: Confirm instrument has gas pressure	Gas cylinder may be turned off or empty	<ol style="list-style-type: none"><li>1. Verify that the cylinder has at least 500 PSI and 30 PSI at the outlet of the regulator. Confirm that all valves between the cylinder and the Ion PGM™ Sequencer are open.</li><li>2. Once you confirm gas pressure leading into the instrument, press <b>Yes</b> to retry verification of gas pressure. If the test continues to fail, contact Technical Support.</li></ol>
Bottle leak check fails	<ul style="list-style-type: none"><li>• Bottle seal is not tight</li><li>• Bottle may be damaged / defective</li></ul>	<ol style="list-style-type: none"><li>1. Finger-tighten the bottles.</li><li>2. If the bottle continues to leak, replace the bottle.</li><li>3. If leak check continues to fail, contact Technical Support.</li></ol>


## Initialization—Auto pH errors

Observation	Possible cause	Recommended action
Error message: Please insert a chip and press <b>Start</b>	Instrument cannot detect the chip in chip socket	<ol style="list-style-type: none"> <li>1. Open the chip clamp and remove the chip.</li> <li>2. Check for debris under the chip or in the chip socket. Remove any debris by rinsing with 18 MΩ water and gently dabbing the socket with a lab wipe tissue. <b>IMPORTANT!</b> Never rub or wipe the socket. Rubbing the socket can damage it and cause it to fail.</li> <li>3. Look for liquid outside the flow cell of the chip: <div data-bbox="971 705 1471 1079" data-label="Image"> </div> </li> <li>4. If you see liquid, replace the chip with a new (unused) one. Wash the new chip once with 100% isopropanol and twice with SEQ Sample Buffer before using. <b>Note:</b> The new chip can be used for sequencing after initialization completes.</li> <li>5. Close the clamp, then press <b>Start</b> to restart the process.</li> <li>6. If the new chip also fails, there could be a problem with the chip socket. Contact Technical Support.</li> </ol>
Error message: Chip calibration failed	<ul style="list-style-type: none"> <li>• Chip not seated in socket correctly</li> <li>• Damaged chip</li> <li>• Loose Sipper</li> </ul>	<p>Follow the procedure for "Error message: Please insert a chip and press Start."</p> <p>Follow the procedure for "Error message: Wash 2 average not stable."</p>


Observation	Possible cause	Recommended action
Error message: The system did not reach the target W2 pH and/or has a clog.	The waste lines may be clogged	<ol style="list-style-type: none"> <li>1. Press the <b>Troubleshoot</b> button.</li> </ol> <p><b>Note:</b> You may choose to skip the <b>Troubleshoot</b> button and change the chip to restart the Auto-pH routine.</p> <ol style="list-style-type: none"> <li>2. Remove the waste bottle.</li> <li>3. Place lab wipes under the waste arm.</li> <li>4. Gently wipe the waste arm with a lab wipe to clear liquid from around the waste line.</li> </ol>  <ol style="list-style-type: none"> <li>5. Press <b>Next</b> to begin buffer flow. Observe flow rates from both waste lines. One line should drip slightly faster than the other. Following the flow rate check, one of 3 results is possible: <ol style="list-style-type: none"> <li>a. If flow rate appears normal, press <b>Cancel</b> and test another chip. If Auto pH failure persists, contact Technical Support.</li> <li>b. If flow is blocked, press <b>Line Clear</b> to run the standard Line Clear procedure. If the line is unable to clear, contact Technical Support.</li> <li>c. If the result of the flow rate check are uncertain, press <b>Re-flow</b> to re-flow the buffer and re-test the flow.</li> </ol> </li> </ol> 



Observation	Possible cause	Recommended action
Error message: The system did not reach the target W2 pH ( <i>continued</i> )	Wash 1 or Wash 2 sipper may be loose	<ol style="list-style-type: none"> <li>1. Loosen the Wash 1 cap and re-tighten the sipper. Since the gas flows when the cap is loose, tighten the sipper as quickly as possible. (The gas is not harmful to the NaOH solution and is not a hazard.)</li> <li>2. Loosen the Wash 2 cap and re-tighten the sipper. Since the gas flows when the cap is loose, tighten the sipper as quickly as possible. (The gas is not harmful to the W2 Solution and is not a hazard.)</li> <li>3. Press <b>Start</b> to re-start the auto-pH process.</li> </ol>
	Forgot to add NaOH to the Wash 1 Bottle	<ol style="list-style-type: none"> <li>1. If there is no NaOH in the Wash 1 Bottle, loosen the cap and add 350 <math>\mu</math>L of 100 mM NaOH to the Wash 1 Bottle. (The flowing gas is not harmful to the NaOH solution and is not a hazard.)</li> <li>2. Recap the bottle and shake gently to mix.</li> <li>3. Press <b>Start</b> to restart auto-pH.</li> </ol>
	Damaged chip	<ol style="list-style-type: none"> <li>1. Replace the chip with a new (unused) one. Insert the chip in the socket, then press <b>Start</b>. <b>Note:</b> The new chip can be used for sequencing after initialization completes.</li> <li>2. If the error persists, there could be a problem with the chip clamp. Contact Technical Support.</li> </ol>

Observation	Possible cause	Recommended action
Error message: W2 average not stable. Try reseating/replacing chip	Reading for W2 solution is not stabilizing quickly enough	<ol style="list-style-type: none"> <li>1. Remove the waste bottle and gently wipe excess fluid from the waste lines with a lab wipe.</li> </ol>  <ol style="list-style-type: none"> <li>2. Check for leaks and reseat the chip (see troubleshooting for "Chip Check" and "Chip calibration" above). Replace the chip with a new (unused) one if needed. <b>Note:</b> The new chip can be used for sequencing after initialization completes.</li> <li>3. Loosen the cap in the W2 position and re-tighten the sipper. Since the gas flows when the cap is loose, tighten the sipper as quickly as possible. (The gas is not harmful and not a hazard.)</li> <li>4. After performing one or more above steps, press <b>Start</b> to re-start auto-pH. If auto-pH fails even after replacing the chip, contact Technical Support and manually adjust the pH of the Wash 2 Bottle as described in Appendix C, "Manually Adjust W2 pH".</li> </ol>
Error message: W2 out of range	<ul style="list-style-type: none"> <li>• Chip measurements very unstable</li> <li>• Chip is damaged</li> </ul>	See troubleshooting tips for "W2 average not stable" above.
Error message: Chip reading inconsistent. Please replace chip and try again	<ul style="list-style-type: none"> <li>• pH response of the chip is not uniform or reliable</li> <li>• Ran out of W3 Solution or volume too low</li> </ul>	<ol style="list-style-type: none"> <li>1. Verify that there is enough W3 Solution (&gt;25 mL) in the Wash 3 Bottle and that the sipper is secure.</li> <li>2. If necessary, loosen the Wash 3 Bottle cap, tighten the sipper, and add more W3 Solution to fill to 50 mL. Since the gas flows when the cap is loose, perform these operations as quickly as possible. (The gas is not harmful to the W3 Solution and is not a hazard.)</li> <li>3. If there is enough W3 Solution, replace the chip with a new (unused) one. Insert the chip in the socket, then press <b>Start</b>. <b>Note:</b> The new chip can be used for sequencing after initialization completes.</li> </ol>

Observation	Possible cause	Recommended action
Error message: Added too much W1 to W2	<ul style="list-style-type: none"> <li>Poor water quality</li> <li>18 MΩ water exposed to air for too long</li> <li>Incorrect solution added to the Wash 2 Bottle</li> <li>Too little NaOH added to Wash 1 Bottle</li> <li>Damaged chip</li> </ul>	<ol style="list-style-type: none"> <li>1. Check whether the water meets the 18 MΩ specification and 100 mM NaOH and W2 Solution were added correctly.</li> <li>2. If solutions are incorrect or water does not meet specifications, correctly prepare the solution(s) and/or use high-quality water. Abort the initialization and restart using correct solutions/water.</li> <li>3. If solutions are correct and water meets specifications, abort the initialization, return to the main menu, and proceed to the next steps.</li> <li>4. Leave the Wash 2 Bottle on the instrument.</li> <li>5. Remove the Wash 1 Bottle, leaving the sipper on the W1 port. Empty the bottle, and rinse the bottle twice with 18 MΩ water.</li> <li>6. Add 350 µL of 100 mM NaOH to the Wash 1 Bottle and reinstall on the instrument.</li> <li>7. Press <b>Initialize</b>, select the kit type, and keep pressing the <b>Next</b> button to skip all bottle prep steps until the instrument begins purging air from the bottle. Then proceed through the touchscreens as normal to complete the initialization.</li> <li>8. The next time you initialize the instrument, add 140 µL of 100 mM NaOH to the Wash 2 Bottle instead of 70 µL. Continue to use this larger volume for subsequent initializations until you receive an "Overshot Target" error message at the first auto-pH iteration, at which point follow the troubleshooting steps in "Error message: The system overshot the target W2 pH. " on page 64 on the following page and then return to adding 70 µL of 100 mM NaOH.</li> <li>9. If you still receive the same initialization error ("Added too much W1 to W2"), contact Technical Support.</li> </ol>
Error message: UNDERSHOT TARGET PH: W2 pH = n.nn Failed	Auto-pH couldn't add enough Wash 1 to the Wash 2 before the maximum iterations, 10, occurred.	<ol style="list-style-type: none"> <li>1. A blockage may have occurred. Follow the procedure for "Error message: There may be a blockage or no NaOH in W1. Please check W1 and run line clear then try again."</li> <li>2. Press <b>Start</b> to re-start auto-pH. If you still get the "Undershot target pH" error, try replacing the chip with a new (unused) chip and restarting auto-pH.</li> </ol> <p><b>Note:</b> The new chip can be used for sequencing after initialization completes.</p>

Observation	Possible cause	Recommended action
Error message: The system overshoot the target W2 pH.	Auto-pH added more NaOH from the Wash 1 Bottle to the Wash 2 Bottle than was needed, and reports the pH value	<ol style="list-style-type: none"> <li>1. Press the <b>Overshoot</b> button to proceed with W2 pH adjustment.</li> <li>2. Unscrew the cap of the Wash 2 Bottle. Without removing the sipper from the bottle, lift the cap high enough to pipette 15 <math>\mu</math>L of 100 mM HCl into the Wash 2 Bottle, close and tighten cap.</li> </ol>  <ol style="list-style-type: none"> <li>3. Press <b>Next</b> to re-pressurize the Wash 2 Bottle and mix the W2 solution.</li> <li>4. Press <b>Start</b> to retry auto-pH.</li> </ol>

## Initialization—Reagent pH verification

Observation	Possible cause	Recommended action
Red failure screen, reagent pH displayed	One or more reagents are not within the target pH	<ol style="list-style-type: none"> <li>1. Press <b>Start</b> to repeat the pH measurements to confirm the measurement.</li> <li>2. If any reagents still fail, try replacing the chip with a new (unused) chip and repeating.</li> </ol> <p><b>Note:</b> The new chip can be used for sequencing after initialization completes.</p> <ol style="list-style-type: none"> <li>3. If any reagents still fail, clean and re-initialize the instrument with fresh reagents and a new chip.</li> </ol>
Red failure screen, reagent pH <i>not</i> displayed	Chip did not calibrate	<ol style="list-style-type: none"> <li>1. Replace the chip with a new (unused) one.</li> </ol> <p><b>Note:</b> The new chip can be used for sequencing after initialization completes.</p> <ol style="list-style-type: none"> <li>2. Press <b>Start</b> to restart the pH measurement.</li> <li>3. If the second test fails, contact Technical Support.</li> </ol>

## Troubleshooting using the controls

Observation	Possible cause	Recommended action
Ion Sphere™ Test Fragments are not present in the Test Fragment Report section of the run report, and library sequencing is poor	<ul style="list-style-type: none"> <li>Poor chip loading</li> <li>Control Ion Sphere™ Particles were not added to the sample</li> </ul>	<ol style="list-style-type: none"> <li>Confirm that the Control Ion Sphere™ Particles (included in this sequencing kit) were added.</li> <li>If controls were added, contact Technical Support.</li> </ol>
Control Ion PGM™ Ion Sphere™ Particles were added, but sample loading is poor	Problems with library or template preparation	Verify the quantity and quality of the library and template preparations.



# Barcoded libraries

This appendix describes how to select and create barcode sets on the Ion PGM™ System for sequencing barcoded libraries.

## Pre-installed barcode sets

The Torrent Suite™ Software includes the pre-installed barcode sets “IonXpress” and “IonXpressRNA.”

When setting up a Planned Run or performing a run, select the appropriate barcode set for your library type as follows:

- **DNA libraries:** Select the “IonXpress” barcode set, which includes all barcodes in the Ion Xpress™ Barcode Adapters 1–96 Kits.
- **RNA libraries prepared using the Ion Total RNA-Seq Kit v2:** Select the “IonXpressRNA” barcode set, which contains all 16 barcodes in the Ion Xpress™ RNA BC01–16 Kit (Cat. no. 4475485).

If you are not using barcodes:

- **DNA libraries:** Leave the Barcode field blank
- **RNA libraries prepared using the Ion Total RNA-Seq Kit v2:** Select “RNA\_Barcode\_None” from the dropdown list. This will ensure that the proper trimming is performed on the resulting sequence when the RNA library does not have a barcode.

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**IMPORTANT!** Do not edit, delete, or modify the pre-installed barcode sets.

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## Select a barcode set for a sequencing run

Select the barcode set in the Torrent Browser when planning the run.

Select the sequencing kits and then hit next.

Sample Preparation Kit (optional):

Library Kit Type: [Details +](#)

Ion Xpress Plus Fragment Library Kit

Template Kit ☒ OneTouch ☐ IonChef:

Ion PGM Template OT2 400 Kit

Sequencing Kit:

Ion PGM Hi-Q Sequencing Kit

Flows:

Control Sequence (optional):

Chip Type (required):

Ion 318™ Chip v2

Barcode Set (optional)

IonXpress\_1-16

☒ Mark as Duplicates Reads

☒ Enable Base Recalibration

☐ Enable Realignment

## Custom barcode sets

You can create custom sets of barcodes as **comma-separated value (.csv) files**, then load these sets onto the Torrent Server for use during sequencing runs.

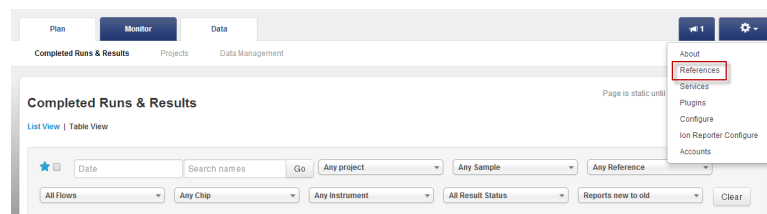
To access the Torrent Server, you must have a username and password. For more information on working with custom barcode sets, refer to the *Torrent Browser User Interface Guide*.

### Create and add a custom barcode set on Ion PGM™ Torrent Server

1. Create a comma-separated variable (CSV) text file for your custom barcode set. The CSV file can contain up to 384 barcodes.

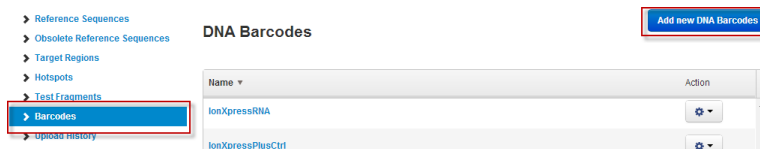
**Note:** You can run fewer than 384 barcodes in a sequencing run; the Ion PGM™ System automatically detects and selects the barcodes used in the run from the selected set.

2. To add the file to the Torrent Server, go to Ion PGM™ Torrent Browser and click the **Gear button** (⚙️) on the right side of the window, then select **References**.

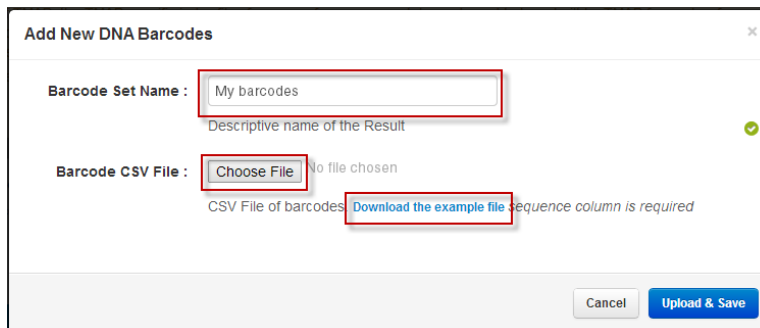


3. In the left navigation bar, select **Barcodes**.

- Click the **Add new DNA Barcodes** button.



- In the popup box, click on the **Download the example file** link for an example file showing the correct CSV format. Edit your own CSV barcode list to match this format, and save the CSV file on your computer.



- Enter the **Barcode Set Name** and click on **Choose File** to select your formatted barcode CSV file. Then click **Upload & Save**.
- The barcode set file name is displayed in the list.

## Other barcode set operations

### View a barcode set

- To view a barcode set, go to the Torrent Browser and click the **References** tab.
- Scroll down to the Barcodes section and click on the barcode set name to display the list of barcodes in the set.

### Delete a custom barcode set from the Torrent Server

- To view the barcode set names, click the **References** tab in the Torrent Browser.
- Scroll down to the Barcodes section and click the name of the barcode set that you want to delete.
- In the barcode set page, click **+ Delete Barcode Set** then click Yes to confirm the deletion.

### Add a barcode to a custom barcode set

- Open the Torrent Browser and click the **References** tab.
- Scroll down to the Barcodes section and click the name of the barcode set to be edited.

3. Click **+ Add Barcode**. You see the new barcode window:

4. Complete the fields, then click **Save Barcode**.

#### Edit or delete a barcode from a set

1. Open the Torrent Browser and click the **Settings** button on the right side of the window, then select **References**.
2. In the Barcodes panel, click the file name of the barcode set to be edited.
3. Click the button under Action to edit or delete the panel.
  - To edit a barcode, change the barcode in the edit window, then click **Save Barcode**.
  - To *delete* a barcode from a set, click **Delete Barcode**, then click **Yes** to confirm the deletion.



# Manually Adjust W2 pH

## Materials and equipment needed

- Orion<sup>®</sup> 3-Star Plus pH Benchtop Meter Kit or equivalent
- Nitrogen gas tank, tube, and flow meter
- 100 mM NaOH (prepared fresh daily)
- Pipette tips and pipette
- Magnetic stirrer and stir bar
- 100 mM HCl

## Procedure

If an error message during the automatic pH process indicates that there is a problem adjusting the pH of the W2 Solution, use the following procedure to manually adjust the pH of the W2 Solution in the Wash 2 Bottle.

1. Before proceeding, rinse an empty Wash 2 Bottle and have it ready next to the instrument. Also have an additional Wash 2 Bottle cap ready.  
**Note:** Gas will be flowing out of the Wash 2 cap, so perform the next steps as quickly as possible (flowing gas will not harm the W2 Solution, and is not a hazard).
2. Remove the Wash 2 Bottle attached to the instrument, and cap the bottle.
3. Secure the empty Wash 2 Bottle (from step 1) to the instrument—do not remove the sipper. This bottle will contain the gas flowing out of the instrument while you pH the W2 Solution and protect the sipper from contamination.
4. Move the Wash 2 Bottle containing the W2 Solution to the stir plate near the nitrogen gas tube.
5. Secure the gas tube so that it extends inside the mouth of the Wash 2 Bottle but not below the surface of the W2 Solution.
6. Set the gas flow to 0.5 lpm. Start mixing the W2 Solution fast enough for a small whirlpool to form.
7. Calibrate the pH meter using a three-point calibration. Rinse any buffering solution from the pH probe prior to preparing solutions.



8. Adjust the pH of the W2 Solution to  $7.55 \pm 0.1$  by adding a small amount of freshly prepared 100 mM NaOH to the solution, and then measuring the pH using the pH meter. Add small aliquots and allow the pH to equilibrate before adding more.

**Note:** If the pH rises above 7.75, use 100 mM hydrochloric acid (HCl) to readjust the pH to  $7.55 \pm 0.1$ .

9. When the pH is stable, turn off the gas, remove the gas line, and cap the Wash 2 Bottle.
10. Move the bottle to the instrument, remove the empty Wash 2 Bottle from the instrument, and place the sipper inside the Wash 2 Bottle whose pH adjusted.
11. Secure the cap firmly. Press **Next** to exit the automated pH check and continue with instrument initialization.



# Sequencing run times

Number of flows	Average read length <sup>[1]</sup>	Average run time by chip type: 314/316/318	Single read runs/kit <sup>[2]</sup>
850	400 bp	4.8 / 6.3 / 9.4 hours	4
500	200 bp	2.4 / 3.1 / 4.5 hours	8
260	100 bp	1.3 / 1.7 / 2.4hours	12

<sup>[1]</sup> Read length may vary based on library size.

<sup>[2]</sup> Only 4 runs are supported for any read length. For best results, run should be started within 1 hour after initialization.



# Ion PGM™ Chip Minifuge

The Ion PGM™ Chip Minifuge is supplied with one custom rotor and two buckets. The buckets are designed to hold two chips: one in each bucket. The rotor and bucket design enables effective and efficient reagent loading of chips.



## Safety precautions



### CAUTION!

- Make sure your supply voltage matches the voltage label on the minifuge, i.e., never plug a 120V minifuge into an 220–240 VAC outlet. Operating the minifuge with a supply voltage outside the specified range may cause a fire or electric shock.
- Do not run the minifuge for more than 30 seconds.
- Never operate the minifuge without a rotor properly attached to the shaft.
- Never operate with only one chip in place. A chip must be present in each bucket to balance the rotor. If necessary, you can balance a loaded chip with a used chip of any type.
- Never put hands in the rotor area unless the rotor is completely stopped.
- Never move the minifuge while the rotor is spinning.
- Do not leave the minifuge running when not in use.

## Voltage selection

Two different minifuges are available, depending on your supply voltage: 120 VAC and 230 VAC. Make sure that the voltage specification on the label of your minifuge matches the supply voltage. If they do not match, change your supply voltage or contact Customer Support to request the appropriate minifuge.



**CAUTION!** Never plug a 120V minifuge into an 220–240 VAC outlet, or vice versa. Operating the minifuge with a supply voltage outside the range specified on the label may cause a fire or electric shock.

## Operation

1. Place the Ion PGM™ Chip Minifuge on a level, clean surface near an accessible power outlet so that the cord and outlet are within easy reach of the operator.
2. Make sure the power switch on the minifuge is in the "off" position.
3. Load a chip into each bucket.

**IMPORTANT!** A chip must be present in each bucket to balance the rotor. If necessary, you can balance a loaded chip with a used chip of any type.

4. Turn the power switch on.
5. To begin centrifugation, close the lid of the minifuge. (The centrifugation time will vary depending on the step in the chip-loading protocol.)
6. To stop centrifugation, press down on the lid release tab on the front of the minifuge.



**CAUTION!** Do not attempt to open the lid or remove the chips until the unit has come to a complete stop.

7. After the rotor has stopped, open the lid by grabbing it with the thumb on the front and fingers on the back then lifting the lid back on the hinge.

## Voltage, RPM, and RCF

The following tables list the revolutions per minute (RPM) and relative centrifugal force (RCF) at different voltages.

120/50 VAC, 60 Hz	RPM	RCF
90	4100	836
100	4550	1030
110	4960	1224

120/50 VAC, 60 Hz	RPM	RCF
120	5330	1424
130	5710	1628

230/50 VAC, 60 Hz	RPM	RCF
210	5070	1279
220	5310	1403
230	5515	1513
240	5705	1619
250	5900	1732

## Cleaning

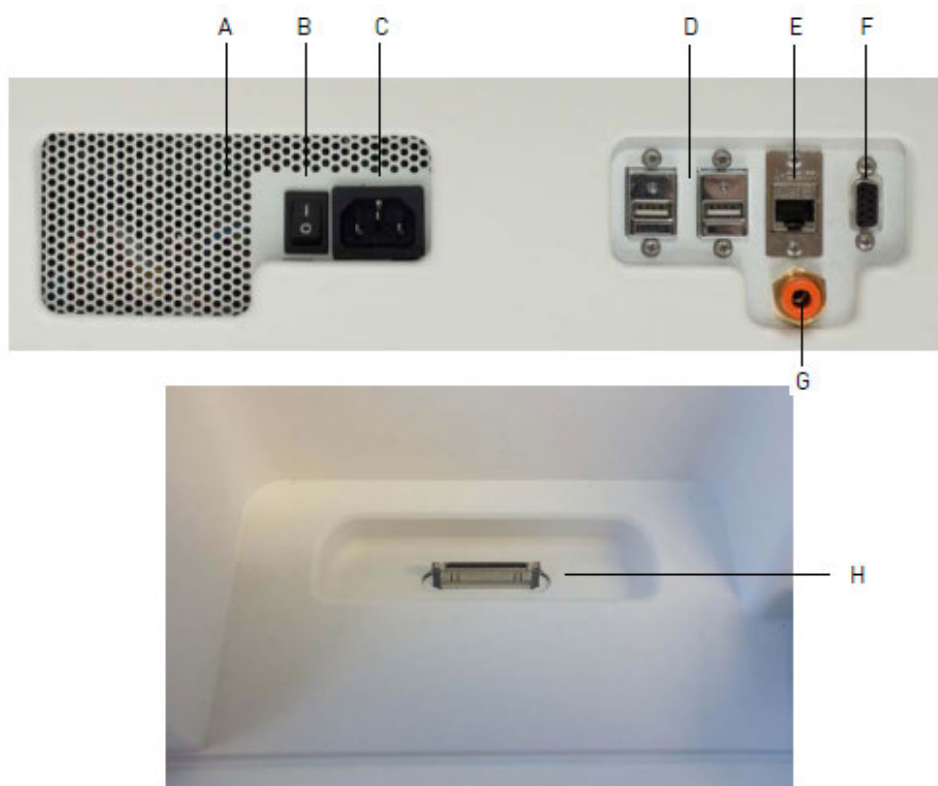
To clean the minifuge, use a damp cloth and a mild, noncorrosive detergent (pH <8). After cleaning, ensure that all parts are dried thoroughly before attempting to operate the unit. Do not immerse the centrifuge in liquid or pour liquids over it.

**Note:** Use only the cleaning protocol described above.



# Additional instrument information

## Ion PGM™ Sequencer input and output connections



Label	Component	Description
A	Instrument fan cover	<b>IMPORTANT!</b> The fan cover must be unobstructed to ensure adequate cooling and proper functioning of the Ion PGM™ Sequencer.
B	On/off switch	Power switch, where the states are on ( I ) or off ( O ).
C	Power port	100-240VAC port that provides power to the instrument.



Label	Component	Description
D	USB ports	Connects the barcode reader to the instrument.
E	Ethernet port	An RJ45 port that provides Ethernet (Gigabit) communication with the Ion PGM™ Sequencer.
F	RS232 port	A diagnostic port
G	Gas inlet	For nitrogen gas.
H	iPod® port	A port for docking your iPod® portable media player

## Power the Ion PGM™ Sequencer on or off

### Power on

**Note:** If the Ion PGM™ Sequencer is powered on, and the touchscreen is blank, touch the screen to "wake" the touchscreen.

1. Locate the power switch on the back of the instrument and turn to the on (I) position.
2. Press the power button on the front of the instrument. The switch should illuminate. When the instrument touchscreen Main Menu appears, the instrument is ready for use.
3. See "Cleaning schedule" on page 23 for when to perform 18 MΩ water or chlorite solution cleaning after powering on.

### Power off

It is not necessary to power off the instrument overnight or over the weekend. If the instrument will not be used for more than 3 days, power off the instrument as follows:

1. In the Main Menu, select **Tools > Shut Down**.
2. If you have not already cleaned the instrument, select 18 MΩ water cleaning, then press **Next** to start the cleaning process.
3. When cleaning is complete, press **Shut Down**.
4. After you exit the main touchscreen, press the **Halt** button, then **OK** when prompted. The instrument will power down.

## Update the Ion PGM™ Sequencer software

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**IMPORTANT!** After updates are installed, the instrument must be restarted. If an update to the Ion PGM™ Sequencer software is available, the red “Alarms and Events” pop-up appears in the touchscreen Main Menu to alert you. Click the red pop-up to see the detailed messages. If a message states **New Software Available**, update the software as follows:

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1. In the Main Menu, select **Options > Updates**.
2. Select the **Released Updates** checkbox, then press **Check**.
3. When the message **Press Update** to begin update process appears, press **Update**.  
**Note:** If the message “All Software Current” appears, press **Back** to return to the Main Menu.
4. When the message “Installing Completed” displays, follow the onscreen prompts to restart the instrument.  
**Note:** In some cases, the instrument restarts automatically after software installation.



# Safety



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**WARNING! GENERAL SAFETY.** Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
  - Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the “Documentation and Support” section in this document.
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## Symbols on this instrument



Symbols may be found on the instrument to warn against potential hazards or convey important safety information. In this document, the hazard symbol is used along with one of the following user attention words:

- **CAUTION!** – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.
- **WARNING!** – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.
- **DANGER!** – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury.

Symbol	English	Français
	Caution, risk of danger Consult the manual for further safety information.	Attention, risque de danger Consulter le manuel pour d'autres renseignements de sécurité.
	Protective conductor terminal (main ground)	Borne de conducteur de protection (mise à la terre principale)
	Do not dispose of this product in unsorted municipal waste   <b>CAUTION!</b> To minimize negative environmental impact from disposal of electronic waste, do not dispose of electronic waste in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provision and contact customer service for information about responsible disposal options.	Ne pas éliminer ce produit avec les déchets usuels non soumis au tri sélectif.   <b>CAUTION!</b> Pour minimiser les conséquences négatives sur l'environnement à la suite de l'élimination de déchets électroniques, ne pas éliminer ce déchet électronique avec les déchets usuels non soumis au tri sélectif. Se conformer aux ordonnances locales sur les déchets municipaux pour les dispositions d'élimination et communiquer avec le service à la clientèle pour des renseignements sur les options d'élimination responsable.

## Safety alerts on this instrument

Additional text may be used with one of the symbols described above when more specific information is needed to avoid exposure to a hazard. See the following table for safety alerts found on the instrument.

English	French translation
 <b>CAUTION! Hazardous chemicals.</b> Read the Safety Data Sheets (SDSs) before handling.	<b>ATTENTION! Produits chimiques dangereux.</b> Lire les fiches signalétiques (FS) avant de manipuler les produits.
 <b>CAUTION! Hazardous waste.</b> Refer to SDS(s) and local regulations for handling and disposal.	<b>ATTENTION! Déchets dangereux.</b> Lire les fiches signalétiques (FS) et la réglementation locale associées à la manipulation et à l'élimination des déchets.


## Safety information for instruments not manufactured by Thermo Fisher Scientific

Some of the accessories provided as part of the instrument system are not designed or built by Thermo Fisher Scientific. Consult the manufacturer's documentation for the information needed for the safe use of these products.

## Instrument safety

### General


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**CAUTION! Do not remove instrument protective covers.** If you remove the protective instrument panels or disable interlock devices, you may be exposed to serious hazards including, but not limited to, severe electrical shock, laser exposure, crushing, or chemical exposure.

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### Physical injury

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**CAUTION! Moving Parts.** Moving parts can crush, pinch and cut. Keep hands clear of moving parts while operating the instrument. Disconnect power before servicing.

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## Electrical



**WARNING! Ensure appropriate electrical supply.** For safe operation of the instrument:

- Plug the system into a properly grounded receptacle with adequate current capacity.
- Ensure the electrical supply is of suitable voltage.
- Never operate the instrument with the ground disconnected. Grounding continuity is required for safe operation of the instrument.



**WARNING! Power Supply Line Cords.** Use properly configured and approved line cords for the power supply in your facility.



**WARNING! Disconnecting Power.** To fully disconnect power either detach or unplug the power cord, positioning the instrument such that the power cord is accessible.

## Cleaning and decontamination



**CAUTION! Cleaning and Decontamination.** Use only the cleaning and decontamination methods specified in the manufacturer's user documentation. It is the responsibility of the operator (or other responsible person) to ensure the following requirements are met:

- No decontamination or cleaning agents are used that could cause a HAZARD as a result of a reaction with parts of the equipment or with material contained in the equipment.
- The instrument is properly decontaminated a) if hazardous material is spilled onto or into the equipment, and/or b) prior to having the instrument serviced at your facility or sending the instrument for repair, maintenance, trade-in, disposal, or termination of a loan (decontamination forms may be requested from customer service).
- Before using any cleaning or decontamination methods (except those recommended by the manufacturer), users should confirm with the manufacturer that the proposed method will not damage the equipment

## Laser



**CAUTION! LASER HAZARD, Bar Code Scanner.** The bar code scanner included with the instrument system is a Class 2 laser. To avoid damage to eyes, do not stare directly into the beam or point into another person's eyes.

## Safety and electromagnetic compatibility (EMC) standards

The instrument design and manufacture complies with the standards and requirements for safety and electromagnetic compatibility as noted in the following table:

## Safety

Reference	Description
EU Directive 2006/95/EC	European Union “Low Voltage Directive”
IEC 61010-1 EN 61010-1 UL 61010-1 CSA C22.2 No. 61010-1	<i>Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1: General requirements</i>
IEC 61010-2-010 EN 61010-2-010	<i>Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-010: Particular requirements for laboratory equipment for the heating of materials</i>

## EMC

Reference	Description
Directive 2004/108/EC	European Union “EMC Directive”
EN 61326-1	<i>Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements – Part 1: General Requirements</i>
FCC Part 15	U.S. Standard “Industrial, Scientific, and Medical Equipment”
AS/NZS 2064	<i>Limits and Methods of Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radiofrequency Equipment</i>
ICES-001, Issue 3	<i>Industrial, Scientific and Medical (ISM) Radio Frequency Generators</i>

## Environmental design

Reference	Description
Directive 2012/19/EU	European Union “WEEE Directive” – Waste electrical and electronic equipment
Directive 2011/65/EU	European Union “RoHS Directive” – Restriction of hazardous substances in electrical and electronic equipment

## Chemical safety



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**WARNING! GENERAL CHEMICAL HANDLING.** To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the “Documentation and Support” section in this document.
  - Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
  - Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
  - Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
  - Handle chemical wastes in a fume hood.
  - Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
  - After emptying a waste container, seal it with the cap provided.
  - Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
  - Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
  - **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.
-

## Biological hazard safety



**WARNING! BIOHAZARD.** Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Safety equipment also may include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:  
[www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf](http://www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf)
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:  
[www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf](http://www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf)



# Documentation and support

## Customer and technical support

Visit [www.lifetechnologies.com/support](http://www.lifetechnologies.com/support) for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
- Order and web support
- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

**Note:** For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

## Limited product warranty

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